Computer Simulation of Excitation-Contraction Coupling in Cardiac Muscle. A Study of the Regulatory Role of Calcium Binding to Troponin C

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Abstract. The influence of a change of troponin concentration as well of a change of binding and dissociation of Ca^{2+} ions to the regulatory protein troponin C on the time course of isometric tension has been studied using a mathematical model developed to investigate excitation-contraction coupling in cardiac muscle cells The numerical simulations show that peak amplitude, rate of force development, time to peak tension and relaxation time depend significantly on the above parameters even in the case when the equilibrium dissociation constant remains unchanged The obtained results might be useful for the planing of new experiments in the view of the fact that no similar data have been reported for cardiac muscle cells as yet

Key words: Excitation-contraction coupling Mammalian cardiac muscle Computer model — Huxley's mathematical approach to the sliding mechanism

Introduction

Numerous mathematical models have been developed to investigate the properties of the Ca^{2+} signalling system as well as the contractile and mechanical processes in the heart muscle (Wong 1971, 1981, Robertson et al 1981, Cannell and Allen 1984, Mihailova and Petrov 1984, DiFrancesco and Noble 1985, Backx et al 1989, Stern and Lakatta 1992, Michailova and Spassov 1992, 1993 Langer and Peskoff 1996) The models could explain, confirm or reject some physiological and pharmacological hypotheses, and simulate different contractile events However, no experimental or theoretical data are available on how the time course of isometric tension depends on changes of troponin concentration as well as on changes of association and dissociation of Ca^{2+} ions with and from the regulatory protein troponin C

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We included the binding and dissociation of Ca^{2+} ions to and from troponin C in the excitation-contraction coupling model (Michailova and Spassov 1992), more over it was assumed that in mammalian cardiac muscle the concentration of Ca^{2+} ions bound to Ca^{2+} specific sites on troponin C could be the activation function in Huxley's mathematical approach to the sliding mechanism (Huxley 1957) We simulated different mechanical cardiac-muscle responses (isometric contractions at different muscle length and frequency of stimulation tension voltage relation ship, tension-duration relationship force-frequency relationship action potential duration tension relationship) to test the correctness of the model (Michailova and Spassov 1992 1993) The obtained qualitative results corroborate our assumption that the concentration of Ca^{2+} ions bound to Ca^{2+} specific sites on troponin C could be an important regulatory factor in actin-myosin interactions and the subsequent production of force in Huxley's mathematical approach to the sliding mechanism

The main purpose of this study was to investigate how changes in troponin concentration and changes of the association and dissociation of Ca^{2+} ions with and from troponin C influence the time course of isometric tension in response to a single and rhythmical applied action potential, using the mathematical model developed by Michailova and Spassov (1992)

Materials and Methods

In cardiac muscle cells depolarization of the sarcolemma causes influx of Ca^{2+} ions through voltage dependent channels and induces the release of larger amounts of stored Ca^{2+} from the sarcoplasmic reticulum by "calcium-induced calcium release" mechanism The released Ca^{2+} together with Ca^{2+} ions entering through the sarcolemma, bind to troponin C and initiate a sequence of protein interactions that permit activation of the actin-myosin cross-bridge cycle, causing contraction

We described these biophysical and physiological processes in living cardiac muscle by a system of equations (Michailova and Spassov 1992 Eqs 1 18) In contrast to Wong (1981) we included in the model the non-linear differential Eq (4) to describe the association and dissociation of Ca^{2+} ions with and from troponin C

$$d\gamma(t)/dt = k_{on}(trop - \gamma(t))X_{SP}(t) - k_{off}\gamma(t) \qquad (4)$$

where $\gamma(t)$ is the concentration of Ca^{2+} ions bound to Ca^{2+} specific sites on troponin C, $X_{SP}(t)$ is the Ca^{2+} concentration in the sarcoplasm *trop* is the troponin concentration, k_{on}, k_{off} are velocity constants

The inclusion of Eq (4) and the use of $\gamma(t)$ as the activation function in Huxley's model enable to study theoretically how changes of the association and dissociation constants of Ca^{2+} binding to troponin C (k_{on}, k_{off}) as well as changes

of troponin concentration (*trop*) influence the time course of isometric tension

Details of the system of equations (1–18) and the values of parameters that provide for optimal performance of the model have been presented in the paper by Michailova and Spassov (1992). Gill's modifications of Runge–Kutta fourth order algorithm (Ralston and Wilf 1960) and Julian's computational procedure (Julian 1969) are used to solve Eqs. (1–18).

A program written in Fortran by the authors is used to obtain the numerical solutions. The simulations were run on a 486 personal computer.

Results

The initial values of the rate constants for Ca^{2+} binding to troponin C ($k_{on} = 0.39$ l/μmol s, $k_{off} = 19.6$/s) were taken from the work of Hollroyde et al. (1980). The concentration of troponin ($trop = 70$ μmol/l) corresponds to that used by Wier and Yue (1986). In the study the value of each of the parameters k_{on}, k_{off} and *trop* was varied at fixed values of all the other parameters used.

Figure 1*a* shows a three dimensional plot of the isometric tension (Pn) as a function of troponin concentration (*trop*) and time (t) in response to a single applied action potential. The model simulations show that a decrease of *trop* leads to a decrease of the peak amplitude (P_{max}) and of the rate of force development (dP/dt) while the time to peak isometric tension ($t_{max} = 0.2$ s) and the relaxation time ($t_r = 0.6$ s) remain almost unchanged. If the muscle is rhythmically stimulated (60/min) a decrease of *trop* leads to a decrease of the steady-state peak amplitude (P_{st}) (Fig. 1*b*).

From the results shown in Fig. 2*a* it follows that P_{max} and t_r increase in response to a single applied stimulus if the on-rate velocity constant (k_{on}) increases. Figure 2*a* also illustrates that the time to peak isometric tension (t_{max}) remains unchanged ($t_{max} = 0.2$ s) for values of k_{on} (0.001 l/μmol s – 1 l/μmol s) and increases to 0.3 s for values of $k_{on} > 1$ l/μmol s. In the case of rhythmical stimulation an increase of k_{on} leads to an increase of the steady state peak amplitude (P_{st}) (Fig. 2*b*). Figure 2*b* also shows that the resting value of the isometric tension ($Pn_0 = 0.25$) is not reached if the value of on rate constant is too high ($k_{on} = 100$ l/μmol s).

A decrease of the off-rate velocity constant (k_{off}) cause an increase of P_{max} and t_r in response to a single stimulus (Fig. 3*a*). For values of k_{off} (10^4/s – 10^2/s) t_{max} remains unchanged ($t_{max} = 0.2$ s), and it increases to 0.3 s for the values of $k_{off} < 10$/s. If the muscle is rhythmically stimulated a decrease of k_{off} leads to an increase of the steady state peak amplitude (P_{st}) (Fig. 3*b*). The results of simulations (Fig. 3*b*) show that the resting value of isometric tension ($Pn_0 = 0.25$) is not reached if the value of off-rate constant is too low ($k_{off} = 0.01$/s).

Figure 4*a* represents a three dimensional plot of the isometric tension (Pn) as a function of the on-rate velocity constant (k_{on}) and time (t) in response to a

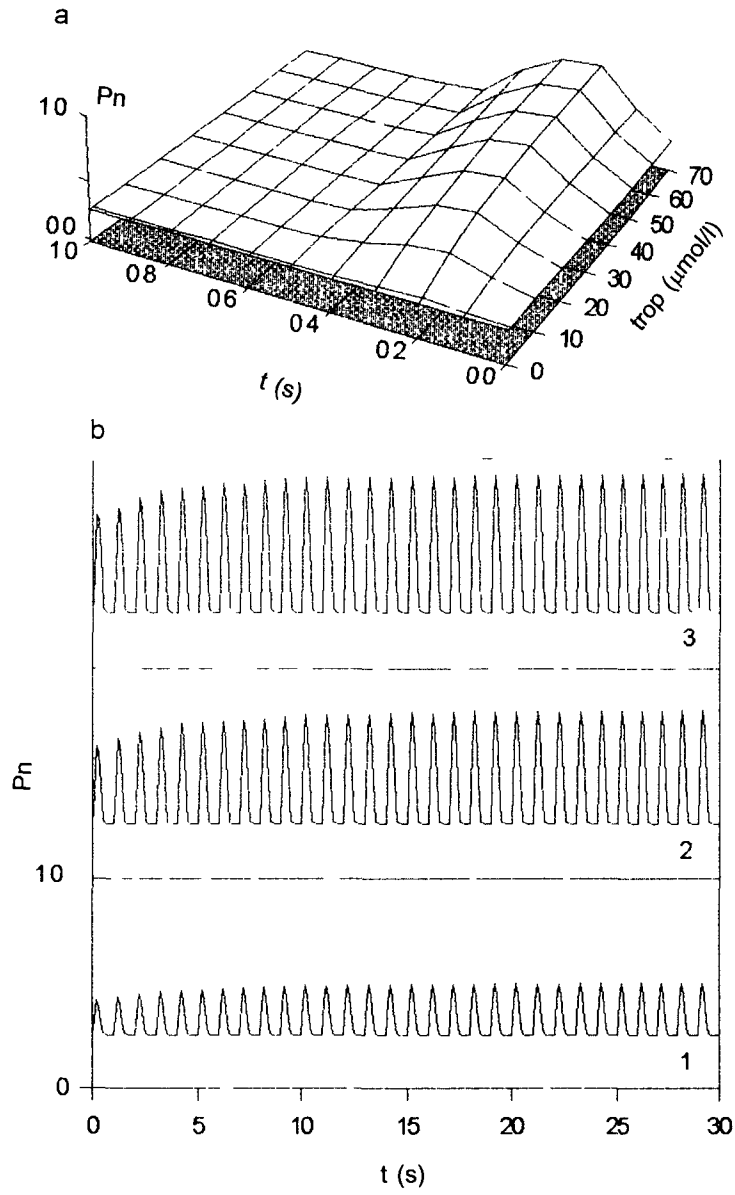

Figure 1. (*a*) Isometric tension as a function of time at different values of troponin concentration (*trop*) in response to a single applied action potential (*b*) Model responses to a rhythmically applied action potential (1) *trop* = 10 μmol/l, (2) *trop* = 40 μmol/l, (3) *trop* = 70 μmol/l *Pn* normalized isometric muscle tension, *t* time Frequency 60/min, k_{on} = 0 39 l/μmol s, k_{off} = 19 6/s

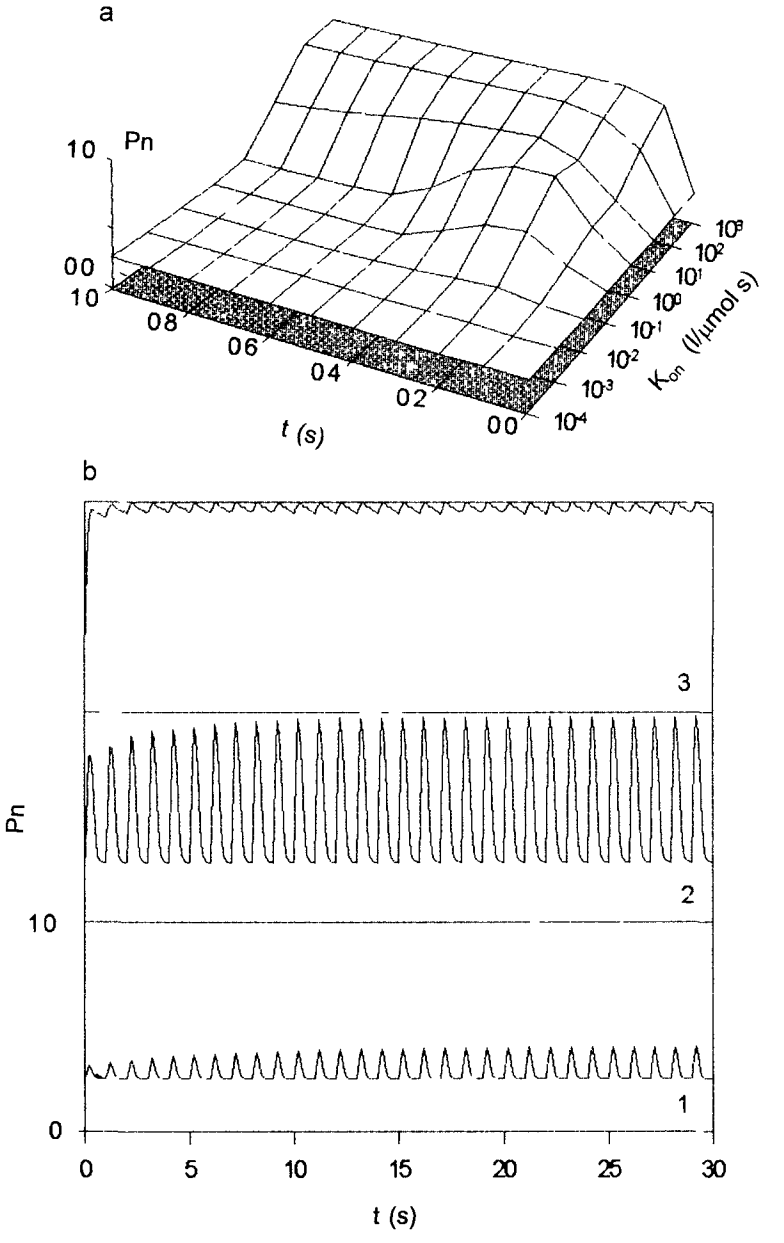

Figure 2. (*a*) Isometric tension as a function of time at different values of the on-rate velocity constant (k_{on}) in response to a single applied action potential (*b*) Model responses to a rhythmically applied action potential (1) k_{on} = 0 01 l/μmol s, (2) k_{on} = 1 l/μmol s, (3) k_{on} = 100 l/μmol s Frequency 60/min *trop* = 70 μmol/l k_{off} = 19 6/s

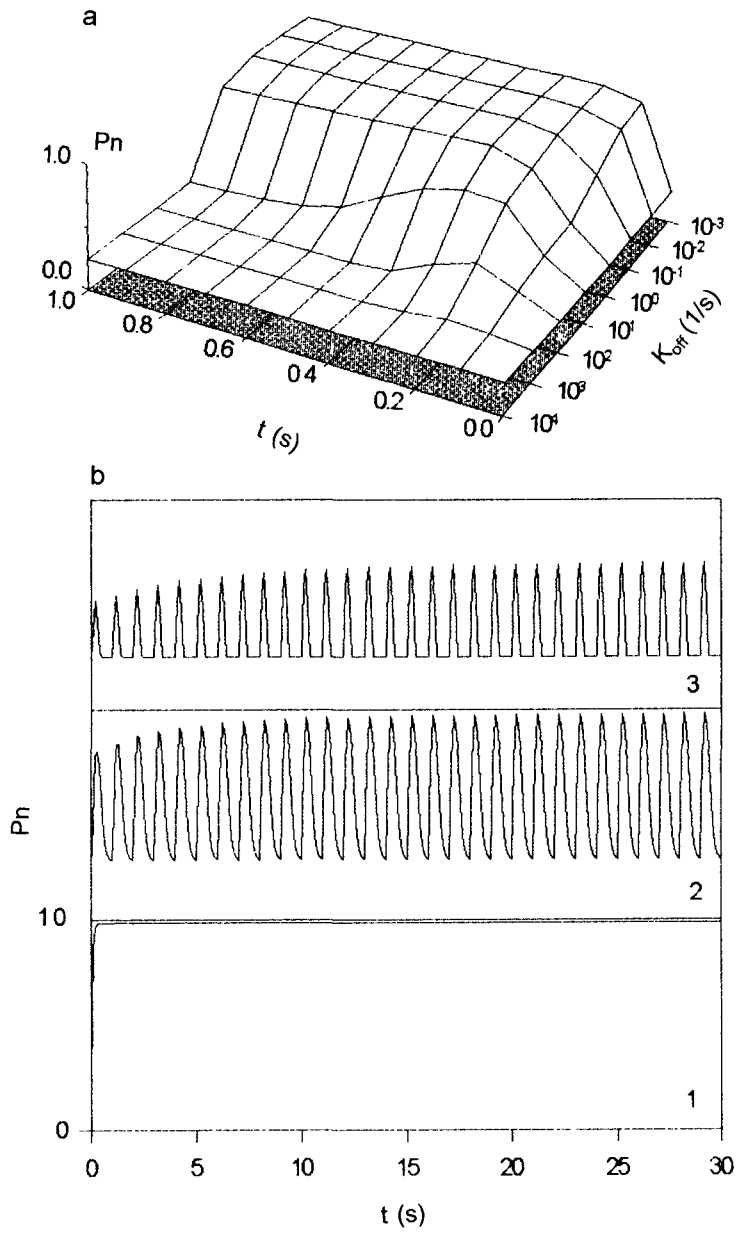

Figure 3. (*a*) Isometric tension as a function of time at different values of the off-rate velocity constant (k_{off}) in response to a single applied action potential. (*b*) Model responses to a rhythmically applied action potential: (1) k_{off} = 0.01/s, (2) k_{off} = 10/s, (3) k_{off} = 100/s. Frequency, 60/min; *trop* = 70 μmol/l; k_{on} = 0.39 l/μmol.s.

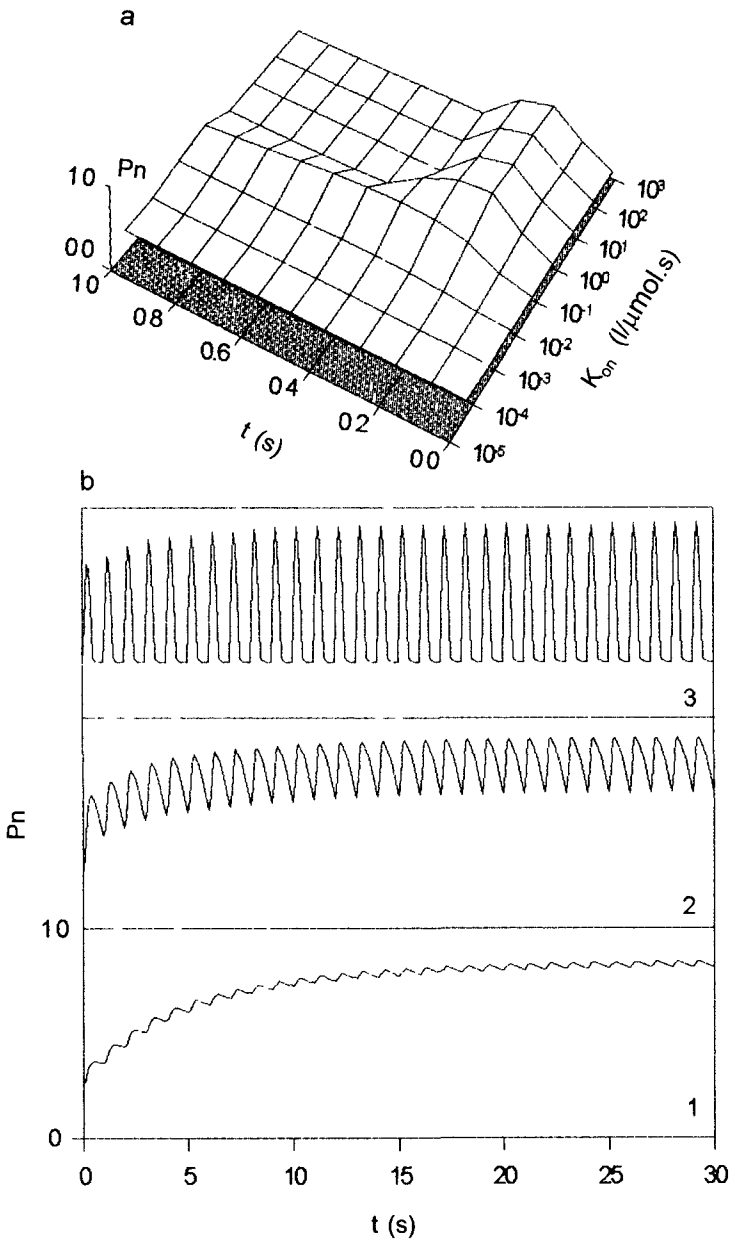

Figure 4. (*a*) Isometric tension as a function of time at different values of the on-rate velocity constant (k_{on}) in response to a single applied action potential with the equilibrium dissociation constant unchanged ($K_d = k_{off}/k_{on} = \text{const}$) (*b*) Model responses to a rhythmically applied action potential and $K_d = \text{const}$ (1) $k_{on} = 0\ 39 \times 10^{-2}$ l/μmol s, $k_{off} = 19\ 6 \times 10^{-2}$/s, (2) $k_{on} = 0\ 39 \times 10^{-1}$ l/μmol s, $k_{off} = 19\ 6 \times 10^{-1}$/s, (3) $k_{on} = 0\ 39$ l/μmol s, $k_{off} = 19\ 6$/s Frequency, 60/min, *trop* = 70 μmol/l

single applied action potential but the equilibrium dissociation constant remains unchanged ($K_d = k_{off}/k_{on}$ = const). An increase of both k_{on} and k_{off} (K_d = const) leads to an increase of the peak amplitude (P_{max}) for values of k_{on} in the interval (0.39×10^{-4} l/μmol s – 0.39 l/μmol s) and k_{off} (19.6×10^{-4}/s – 19.6/s) and to a decrease of P_{max} for values of $k_{on} > 0.391$/μmol s and $k_{off} > 19.6$/s (Fig. 4*a*). The time to peak isometric tension decreases to $t_{max} = 0.2$ s for values of k_{on} (0.39×10^{-4} l/μmol s – 0.39 l/μmol s) and k_{off} (19.6×10^{-4}/s – 19.6/s), and remains unchanged ($t_{max} = 0.2$ s) if $k_{on} > 0.39$ l/μmol s and $k_{off} > 19.6$/s. Figure 4a also shows that t_r considerably decreases when $k_{on} > 0.39$ l/μmol s. If the muscle is rhythmically stimulated the steady-state peak amplitude (P_{st}) increases for values of k_{on} (0.39×10^{-2} l/μmol s – 0.39 l/μmol s) and k_{off} (19.6×10^{-2}/s – 19.6/s). The resting value of isometric tension ($Pn_0 = 0.25$) could not be reached for the lower values of the rate constants ($k_{on} = 0.39 \times 10^{-2}$ l/μmol s, $k_{off} = 19.6 \times 10^{-2}$/s and $k_{on} = 0.39 \times 10^{-1}$ l/μmol s, $k_{off} = 19.6 \times 10^{-1}$/s).

Discussion

The model results (Figs. 1–3) suggest that the characteristics of the isometric tension (peak amplitude, rate of force development, time to peak tension, relaxation time) undergo significant changes if the troponin concentration (*trop*) and the association and dissociation constants of Ca^{2+} binding to troponin C (k_{on}, k_{off}) are varied. An interesting model result (Fig. 4) is also that the characteristics of isometric tension depend significantly on the values of the rate constants (k_{on}, k_{off}) even at unchanged value of the equilibrium dissociation constant ($K_d = k_{off}/k_{on}$ = const).

These theoretical predictions could help to throw more light upon the dependence of excitation-contraction coupling on the Ca^{2+} binding to troponin C and could be useful to plan new experiments in the view of the fact that such data have not yet been reported for cardiac muscle cells. They also may serve as reference point in efforts to explain the reasons for some muscle diseases or the effects of action of several pharmacological agents. For example if it were possible to block a part of the specific calcium binding sites on troponin C, the effect is expected to be mainly on the magnitude of the developed muscle force (Fig. 1). However variations of the rate constants due to possible structural or environmental changes show a more complicated picture: the effect is not only on the peak amplitude of tension but also on the rate of force development and on the peak and the relaxation time (Figs. 2–4).

The dependence of the time course of isometric tension on changes of k_{on}, k_{off}, *trop* and K_d in response to a single and a rhythmical applied action potential cannot be simulated with the known models (Wong 1971, 1981, Robertson et al. 1981, Cannell and Allen 1984, Mihailova and Petrov 1984, DiFrancesco and Noble 1985, Backx et al. 1989, Stern and Lakatta 1992, Langer and Peskoff 1996).

It is important to stress that cardiac cell is not homogenous and the spatial gradients of free Ca^{2+} can thus affect the calculated force time course In recent years some aspects of the subcellular Ca^{2+} concentration gradients have been addressed in computer models of cardiac excitation-contraction coupling (Backx et al 1989 Stern and Lakatta 1992 Amstutz et al 1996 Langer and Peskoff 1996) We do not see any principal difficulties in the future to extend the present model taking into account not only time dependence but also three dimensional distribution of calcium concentration

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References

Amstutz C Michailova A Niggli L (1996) The role of local events and diffusion in cardiac EC-coupling Biophys J **70**, A274

Backx P H De Tombe P P Van Deen J H K Mulder B J M Ter Keurs H E D J (1989) A model of propagating calcium induced calcium release mediated by calcium diffusion J Gen Physiol **93** 963 977

Cannel M B Allen D G (1984) Model of calcium movements during activation in the sarcomere of frog skeletal muscle Biophys J **45** 913 925

DiFrancesco D Noble D (1985) A model of cardiac electrical activity incorporating ionic pumps and concentration changes Phil Trans Roy Soc London **307** 353 398

Holroyde M J Robertson S P Johnson J D Solaro R J Potter J D (1980) The calcium and magnesium binding sites on cardiac troponin and their role in the regulation of myofibrillar adenosine triphosphatase J Biol Chem **255** 11688 11693

Huxley A F (1957) Muscle structure and theories of contraction Progr Biophys Chem **7** 255—318

Julian F J (1969) Activation in a skeletal muscle contraction model with a modification for insect fibrilar muscle Biophys J **9** 547 570

Langer G Peskoff A (1996) Calcium concentration and movements in the diadic cleft space of the cardiac ventricular cell Biophys J **70** 1169—1182

Michailova A P Spassov V Z (1992) Theoretical model and computer simulation of excitation-contraction coupling of mammalian cardiac muscle J Mol Cell Cardiol **24** 97 104

Michailova A P Spassov V Z (1993) Computer simulation of electrical mechanical and molecular processes in mammalian cardiac muscle In Mathematical Modelling Methodology Software Tools and Applications (Ed I Popova) DATECS Publ Sofia Bulgaria pp 121 124

Mihailova A P Petrov N K (1984) Mechano mathematical model of excitation-contraction coupling in muscle tissue Biotheology Suppl I 209 212

Ralston A Wilf H F (Eds) (1960) Mathematical Method for Digital Computers Wiley New York

Robertson S P Johnson J D Potter J D (1981) The time course of Ca^{2+} exchange with calmodulin troponin parvalbumin and myosin in response to transient increases in Ca^{2+} Biophys J **34** 559—569

Stern M D Lakatta E G (1992) Excitation-contraction coupling in the heart the state of the question FASEB J **6**, 3092 3100

Wier W G Yue D T (1986) Intracellular calcium transients underlying the short term force-interval relationship in ferret myocardium J Physiol London **376** 507 530

Wong A Y K (1971) Mechanics of cardiac muscle based on Huxley's model mathematical simulation of isometric contraction J Biomech **4** 529 540

Wong A Y K (1981) A model of excitation-contraction coupling of mammalian cardiac muscle J Theor Biol **90** 37 61

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