Simulation by Two Calcium Store Models of Myocardial Dynamic Properties: Potentiation, Staircase, and Biphasic Tension Development

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Abstract. Most considerations and models concerning myocardial dynamic properties e.g. potentiation and staircase, are based upon the existence of storage structures in the heart muscle cell. The phenomenon of biphasic tension development (or two-component contraction) in heart muscle preparations of several mammalian species suggests that the sarcoplasmic reticulum is one, but by no means the major, source of activator calcium for the contractile system. The simulation of dynamic properties including biphasic tension development was performed in two steps by a simple „two-Ca store-model" and by an "expanded two-Ca store-model" with following results: 1. Increasing potentiation indicated a decrease in the degree of coupling between the Ca stores. A shift of the interval strength curve to lower intervals as well as a decrease of the steady state contraction height implies a decrease of both, the coupling and the leakage time constant. There was no standard relation between staircase phenomena and structure parameters. 2. Analog displays showed a late (or second) component at prolongated stimulation intervals, in the transient phase after a rest period, in the case of perfectly coupled or uncoupled stores, and at great time constant τ_p (which characterizes the calcium pump activity). It is concluded that the late component of biphasic tension development is due to direct activation by the transsarcolemmal Ca flux of the myofilaments, whereas the early component is caused by the release of stored calcium. In the absence of an early component neither potentiation nor marked treppe may be expected.

Key words: Ca stores — Degree of coupling — Potentiation — Staircase — Biphasic tension development

Introduction

There is general agreement that calcium being located in several organelles of the mammalian myocardial cell influences strength and form of tension development

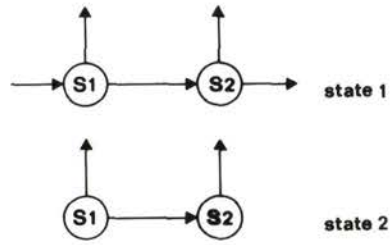

Fig. 1. Two-Ca store-model during state 1 and state 2. For explanation see the text.

(Morad and Goldman 1973; Fabiato and Fabiato 1979; Langer et al. 1982). Each deviation from a constant beat sequence of the rhythmically stimulated heart muscle results in a transient change of subsequent contractions which probably is due to redistribution of calcium in compartments including sarcolemma, sarcoplasmic reticulum, mitochondria, and cytosol (Kruta and Bravený 1968; Wussling and Szymanski 1973; Allen et al. 1976; Beresevicz and Reuter 1977; Langer et al. 1979). The mammalian myocardium usually shows staircase phenomena which often are accompanied by potentiation effects. Most considerations and models concerning myocardial dynamic properties are based upon the existence of calcium storage structures in the heart muscle cell (Kruta and Bravený 1968; Wussling and Szymanski 1972; Morad and Goldman 1973; Kaufmann et al. 1974; Edman and Johannsson 1976; Koester 1979; Nayler and Grinwald 1981; Szymanski and Wussling 1984). To get insight into potentiation and staircase phenomena as well as biphasic tension development (Morad and Goldman 1973; Reiter et al. 1984b) the properties of a two-Ca store-model and those of a model consisting of two Ca stores in cooperation with the contractile element were investigated.

Models and their properties

I. The two-Ca store-model

Fig. 1 explains the function of the "two-Ca store-model" (Wussling and Szymanski 1972). During the action potential (state 1) calcium enters the heart cell and is taken up by the store S1. At the same time calcium is completely released from store S2. The upward arrows point to calcium leakages. During the rest interval between two action potentials (state 2) calcium exclusively moves between the stores and out of the stores via leakages.

We shall denote τ_1 the time constant which characterizes the filling process during state 1 of S1. To simplify the mathematical description, the model is assumed to consist of two equal compartments, the coupling of which is dependent on the time constants τ_K (coupling time constant) and τ_L (leakage time constant). The latter is thought to be equal for each compartment. x_1 and x_2 stand for Ca

concentrations in S1 and S2, respectively (for the mathematical description of the model in detail, see Wussling 1979).

In state 1 the system behaves according to the differential equation

$$dx_1/dt + (\tau_1^{-1} + \tau_K^{-1} + \tau_L^{-1})x_1 = \tau_1^{-1}x_e \tag{1}$$

where x_e is considered to be analog to the calcium concentration in the extracellular space. The general solution is

$$x_1 = \frac{\tau_1^{-1}}{\tau_1^{-1} + \tau_K^{-1} + \tau_L^{-1}} x_e(1 - e^{-(\tau_1^{-1} + \tau_K^{-1} + \tau_L^{-1})t}) + x_1(0)e^{-(\tau_1^{-1} + \tau_K^{-1} + \tau_L^{-1})t} \tag{2}$$

$x_1(0)$ is the Ca concentration in S1 at the start of state 1. In state 2, the following differential equations hold

$$\begin{aligned} dx_1/dt &= -(\tau_K^{-1} + \tau_L^{-1})x_1 + \tau_K^{-1}x_2 \\ dx_2/dt &= \tau_K^{-1}x_1 - (\tau_K^{-1} + \tau_L^{-1})x_2 \end{aligned} \tag{3}$$

With the initial conditions $x_1(0) = x_{10}$ and $x_2(0) = 0$ the solutions are

$$\begin{aligned} x_1 &= 0.5\ x_{10}e^{-\tau_L^{-1}t} \quad (1 + e^{-2\tau_K^{-1}t}) \\ x_2 &= 0.5\ x_{10}e^{-\tau_L^{-1}t} \quad (1 - e^{-2\tau_K^{-1}t}) \end{aligned} \tag{4}$$

The interaction of S1 and S2 is given by the degree of coupling q (Wussling and Szymanski 1980) with

$$q = \tau_L/(\tau_K + \tau_L). \tag{5}$$

q varies between 0 (stores uncoupled) and 1 (stores maximally coupled).

Taking into consideration solutions (2) and (4) the properties of the two-Ca store-model may be studied in correspondence to experimental procedures as applied to myocardial preparations. Accordingly, (i) potentiation, (ii) interval strength relation, (iii) steady state contraction, and (iv) staircase phenomena shall be considered in terms of the model. The plots shown in Figs. 2 to 7 are based on a detailed mathematical description of the model described previously (Wussling and Szymanski 1973; Wussling 1979).

(i) Potentiation

It is well known that the first contraction after rest may be enhanced in comparison to the steady state contraction. Let potentiation be the ratio of the maximal post-rest contraction and steady state contraction and let the twitch tension be roughly proportional to the calcium concentraion x_2 immediately prior to the release (Morad and Goldman 1973). According to the model, potentiation P is the ratio of maximum concentration and steady state concentration in calcium store 2 immediately before the release. We denote the steady state interval t and the rest duration belonging to a maximal post rest contraction t_{max}. Let $t/t_{max} = T$. Fig. 2 shows P in dependence on the degree of coupling $q(0 < q < 1)$ using T as

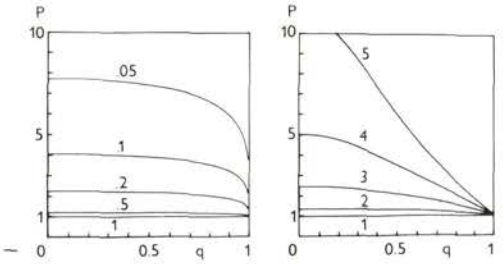

Fig. 2. Dependence of potentiation, P (see text) upon the degree of coupling, q (see eq. (5)). Parameter $T = t/t_{max}$ (t = steady state interval, t_{max} = rest period of maximal Ca concentration in S2). $T \leqq 1$ (left panel) and $T \geqq 1$ (right panel).

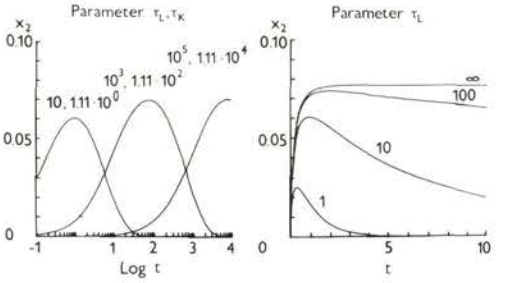

Fig. 3. Interval strength curves (t = steady state interval, x_2 = Ca concentration in S2 prior to release). Parameter and t in seconds, x_2 in arbitrary units. Duration of state 1 = 0.1 s, $\tau_1 = 1$ s (for τ_1 see text). q constant (0.9) in the left part, q varied (with $\tau_K = 1$ s) in the right panel. Note the logarithmic scale of t in the left panel.

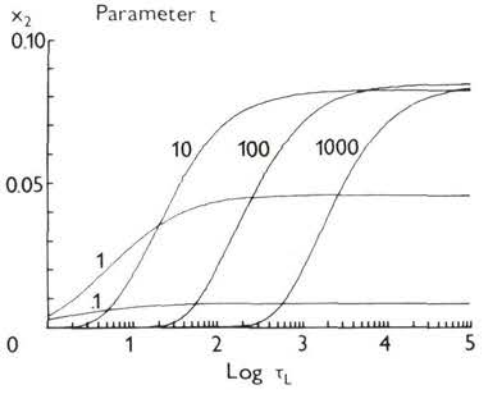

Fig. 4. Steady state Ca concentration x_2 (arbitrary units) prior to release against leak time constant τ_L (seconds). The parameter steady state interval in seconds. Duration of state 1 = 0.1 s, $\tau_1 = 1$ s, $\tau_K = 10$ s.

a parameter. Obviously decreasing coupling results in greater potentiation. At the left part of Fig. 2, curves are plotted for ratios $T<1$. P rises with decreasing t. As a matter of fact, potentiation in the proper sense disappears for $T=1$, i.e. when the steady state interval is equal to the interval of maximum contraction ($t=t_{max}$); however, according to the former definition, this makes $P=1$. On the other hand, for $T>1$, $P>1$ (right part of Fig. 2). In other words, the maximum contraction develops within the steady state interval.

(ii) Interval strength relation

Corresponding plots resulting from the two store model are shown in Fig. 3. The left part of Fig. 3 shows the effects of the time constants τ_L and τ_K which differ by the factor of 10^2 from curve to curve, while the degree of coupling remains the same ($q=0.9$). In the right part of Fig. 3, τ_L is parameter and $\tau_K=1$ s. According to eq. (5) q increases with increasing τ_L. If there are no leakages (τ_L infinite) no maximum exists, i.e. x_2 approaches the greatest value asymptotically.

(iii) Steady state contraction

The contraction height under steady state conditions is the greater the greater the degree of coupling between the calcium stores.

If the steady state interval t is parameter the model supplies a family of curves which approach quite different values of x_2 with the increasing leakage time constant τ_L (Fig. 4). An increase in τ_L results in an increase in q (eq. (5), τ_K constant). Fig. 4 thus clearly confirms the above statement (iii). On the contrary, Fig. 5 seems to suggest a nonunique relation between developed tension and q. In Fig. 5 the leakage time constant was set to 100 s and infinite, respectively (compare plots to the left and right). The reason for the nonunique relation between x_2 and q is that a decrease in τ_K (increase in q) results in an increase in the direct calcium influx thus reducing the amount of Ca^{2+} in the stores. It should be emphasized that in all the calculations based on the two-Ca store-model the direct calcium influx was neglected.

(iv) Staircase phenomena

Let x_{21} be the calcium concentration in store 2 of the first beat during the interval step-function response or during the transient phase after a rest (exactly, after the post-rest beat). Further, let x_2 be the calcium concentration in store 2 after the end of the transient response. The ratio x_{21}/x_2 seems to be a convenient quantity to characterize the dynamic properties of the two store system. Fig. 6 shows x_{21}/x_2 plotted against the time constants τ_K (with $\tau_L=100$ s) and τ_L (with $\tau_K=10$ s), respectively. The steady state interval prior to the step was 1 s in all cases whereas the new intervals are indicated at the curves. From Fig. 6 it is evident that the range with optimal dynamic properties (i.e. the range with distinct staircase phenomena

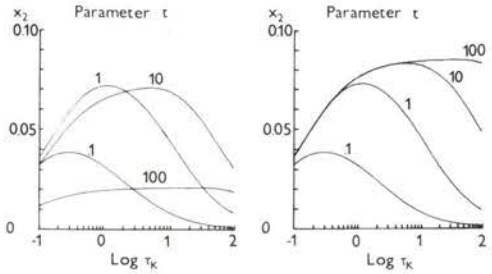

Fig. 5. Steady state Ca concentration x_2 (arbitrary units) prior to release against coupling time constant τ_K (seconds). The parameter steady state interval in seconds. Duration of state $1 = 0.1$ s, $\tau_1 = 1$ s, $\tau_L = 100$ s (left panel) and infinite (right panel).

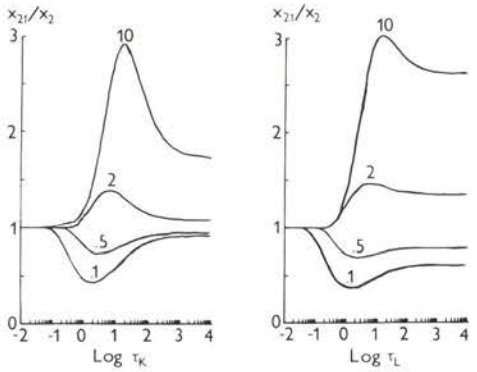

Fig. 6. Staircase after interval steps. Plots of x_{21}/x_2 against coupling time constant τ_K (left panel, $\tau_K = 100$ s) and leak time constant τ_L (right panel, $\tau_K = 10$ s). x_{21} Ca concentration in S2 immediately after a step of interval from 1 s to a value indicated at the curves. x_2 new steady state concentration of S2. Duration of state $1 = 0.1$ s, $\tau_1 = 1$ s.

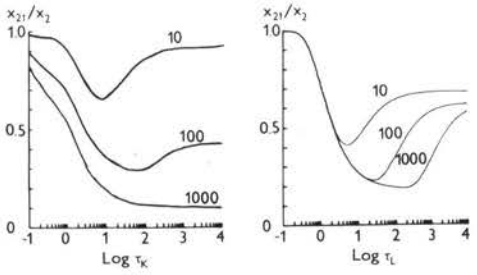

Fig. 7. Staircase after rest. x_{21} = Ca concentration in S2 at the begin of the positive treppe. x_2 = steady state value. Rest periods indicated at the curves. For other values see legend to Fig. 6.

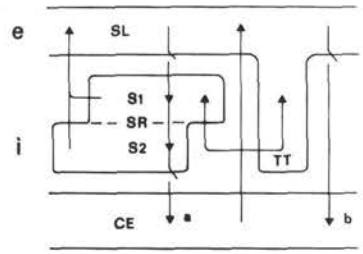

Fig. 8. Schematic representation of the expanded two-Ca store-model. For explanation see text.

may be observed) is determined by the time constants. If both τ_K and τ_L approach to zero the transition phase disappears. Evidently, if τ_K approaches infinite (uncoupled stores) this phase disappears as well. Fig. 7 also reflects these properties to a certain extent. Here, x_{21} means the first beat of a positive treppe after a rest with a duration indicated in seconds. The plots of x_{21}/x_2 against τ_K (with $\tau_L = 100$ s) and τ_L (with $\tau_K = 10$ s), respectively, show different dynamic ranges depending on the chosen resting time. Staircase phenomena may undoubtedly be explained on the assumption of the existence of a two calcium store system. From Figs. 6 and 7 it follows that these phenomena disappear if q approximates 1 or 0. Therefore, the appearance or the missing of staircase phenomena says nothing about the degree of coupling.

II. The two-Ca store-system in relation to the contractile element: expanded two-Ca store-model

The contraction-relaxation cycle results from the cooperation of sarcolemma (SL), sarcoplasmic reticulum (SR), and the contractile element (CE). In the scheme in Fig. 8 the arrows indicate the direction of calcium movements. The intracellular space (i) is bordered by SL from the extracellular space (e). Voltage activated Ca^{2+}-selective channels in the myocardium are considered to be one of the possible routes of Ca^{2+}-entry. Slow channels which enable the extracellular space to be linked up with the intracellular space respecting Ca^{2+} are represented by closed or opened contacts (see Fig. 8). One contact is thought to simulate Ca^{2+} release from the SR. Pathways which involve SR and the plasmalemma must exist to facilitate the sequestration of calcium from the myofilaments and adjacent cytosol (Martonosi 1980). It should be emphasized that, in the scheme, the sarcoplasmic reticulum is composed of two coupled parts, S_1 and S_2, and that during the action potential Ca^{2+} reaches the contractile system directly (b) as well as indirectly (a). Arrows in the schematic representation of calcium movements (Fig. 8) are directed from CE into the extracellular space including transversal tubuli (TT) as well as from the CE to S_1, and from the SR to the extracellular space. The sarcolemmal glycocalyx at the cell surface and the binding sites in the lipid bilayer seem to have a considerable capacity to bind calcium ions and to reach rapid equilibrium with the

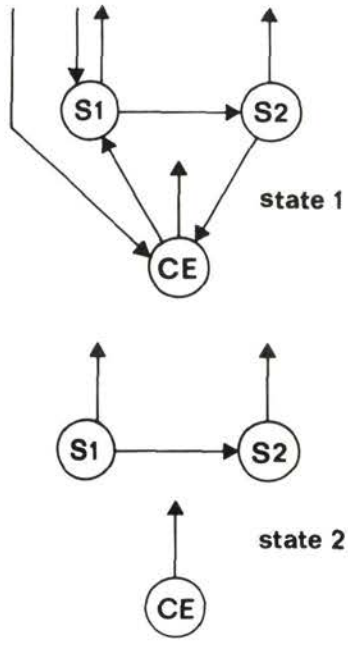

Fig. 9. Expanded two-Ca store-model during state 1 and state 2. For explanation see text.

extracellular space (Langer 1980) so that fluctuations in calcium concentration in this compartment may be neglected. If the supply of calcium in the extracellular space is considered to be unfailing the model still containing two Ca stores may be simplified. Fig. 9 illustrates the function of the "expanded two-Ca store-model".

In state 1 (during the action potential), extracellular calcium enters the cell and intracellular calcium is released from store S2. One part of the extracellular calcium reaches the myofilaments (CE) directly while another part is moving into store S1. Both of them, S1 and S2, are coupled together and provided with leakages. A comparison of Figs. 9 and 1 shows that in state 2, the function of the expanded model is similar to that of the simple two-Ca store-model. The amount of calcium released from SR depends on different factors (e.g. the sarcoplasmic and cytosolic calcium concentrations). Inasmuch as the calcium of S2 may not be released completely certain changes with regard to the dynamic properties are expected to occur in the "expanded two-Ca store-model" as compared to the "two-Ca store-model", without any loss of the principal properties, such as staircase and potentiation.

Figures 10—13 show simulations of the calcium concentration in the CE compartment using an electrical hardware analog.

a) Fig. 10 shows the occurrence of two components during state 1 (see Fig. 9) in

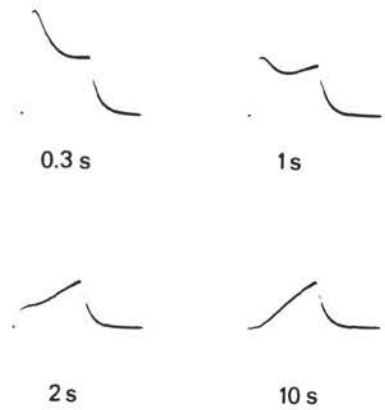

Fig. 10. Analog simulations of Ca concentration in CE at different steady state intervals (0.3 s; 1 s; 2 s; 10 s). Duration of state 1 (0.1 s) from the start to the steep decay.

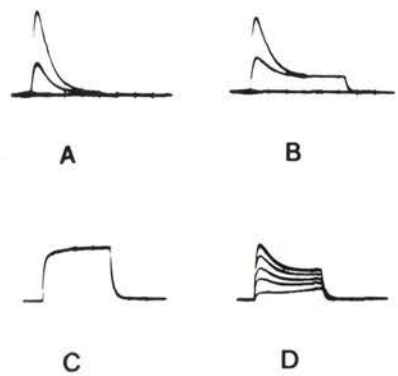

Fig. 11. Simulation of potentiation and staircase. Steady state interval 1 s, rest period 4 s (*A*, *B*, *C*) and 10 s (*D*). Contribution of tonic phase approximately in relation 1 (*A*):10 (*B*):30 (*C*). In D from bottom to top 1st, 2nd, 3rd, 6th, 10th, and 20th (similar to the 10th) post rest trace.

dependence upon the steady state interval. The parameters of the analog remained unchanged. At a short interval, an early component which represents the contribution of store S2 is prevailing. This component disappears after an appropriate prolongation of the interval so that only the late component remains visible. The late component is analogous to that portion of the extracellular calcium which reaches the myofilaments directly.

b) Fig. 11 illustrates the simulation of potentiation and staircase phenomena. The upper curves (Fig. 11*A*, *B*) illustrate potentiation due to a 4 s rest (steady state interval 1 s, cf. lower curves). Both traces show dominating early components suggesting the importance of store S2 with respect to potentiation. The late

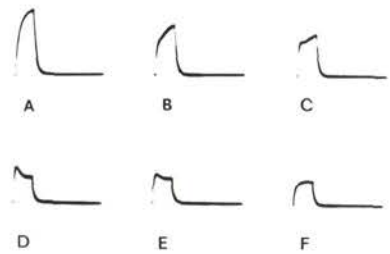

Fig. 12. Influence of coupling between the stores upon analog simulations of Ca concentration in CE. Coupling time constants τ_K:0(*A*), 50 ms (*B*), 100 ms (*C*), 1.5 s (*D*), 2.5 s (*E*), 25 s (*F*). Steady state interval 1 s throughout.

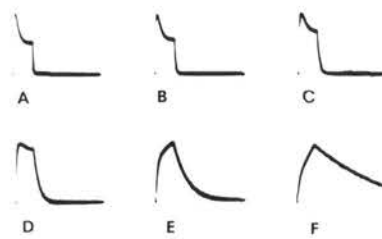

Fig. 13. Influence of time constant τ_P (for explanation see text) upon analog simulations of Ca concentration in CE. τ_P: 10 ms (*A*), 20 ms (*B*), 50 ms (*C*), 100 ms (*D*), 1 s (*E*), 2.5 s (*F*). Steady state interval 1 s throughout.

component (tonic phase) changed in a ratio of 1:10:30 (Fig. 11*A*, *B*, *C*). It is evident that in the absence of the early component no potentiation may be observed, steady state display and that after a pause of 4 s are practically identical (Fig. 11*C*). Finally, Fig. 11*D* shows a family of curves analogous to a positive treppe of the myocard after a post-rest beat. From bottom to top, the 1th, 2nd, 3rd, 6th, 10th, and 20th (similar to the 10th) analog responses after a rest of 10 seconds (steady state interval 1 s). The gradual development of the early component, which becomes dominant during the positive treppe indicates the filling of store S2. It is obvious that both the early and the late component reach their maximum at steady state.

c) The occurrence of one or two components is determined by the coupling time constant τ_K. If a constant storage capacity is supposed and only τ_K is varied the expanded two-Ca store-model shows properties which clearly include those of the two-Ca store-model. There is no unique relation between the calcium amount released from store S2 and τ_K according to the two-Ca store-model (see Fig. 5). From Fig. 12*A*—*F* it is evident that, as simulated by the expanded model, the

calcium concentration in the CE-compartment decreases monotonously with increasing τ_K. The contribution of S_2 is minimal when τ_K approximates zero (compare Fig. 5 and Fig. 12*A*) but the area bordered by the display (Fig. 12*A*) and the zero line is maximal due to the direct contribution of S_2. $\tau_K = 0$ means that both stores are joined. In Fig. 12*B*, *C* ($\tau_K = 50$ ms and $\tau_K = 100$ ms, respectively) the early component is more or less clearly visible and the late component is dominant. On the other hand, Fig. 12*D*, E ($\tau_K = 1.5$ s and $\tau_K = 2.5$ s, respectively) shows a smaller late component. A very large coupling time constant means that the stores are almost entirely isolated. In this case, the contribution of store S2 is covered by the late component (Fig. 12*F*, $\tau_K = 25$ s). Because of the interval dependence of the early component (see Fig. 10) the steady state time interval was 1 s throughout.

d) Let τ_P be a time constant characterizing the decrease of the CE—Ca^{2+} concentration through pathways which involve the sarcoplasmic reticulum and Ca^{2+}-activated ATPase of the sarcolemma (calcium pumps). These pathways should facilitate the retrieval of Ca^{2+} from the CE compartment for the restoration of Ca^{2+} in S1, and for the expulsion from the cell of Ca^{2+} that had entered it during the slow inward current (see schematic representation, Fig. 8). The expanded model combines these and other possible events assuming only one time constant, τ_P. Obviously, the behaviour of the curves simulating the CE—Ca^{2+} concentration strongly depends on τ_P (Fig. 13*A*—*F*). The appearance of the early component is most probable at a small τ_P (see Fig. 13*A*—*C*, τ_P was chosen 10 ms, 20 ms and 50 ms, respectively), i.e. in the case of a high activity of Ca^{2+} pump. Both components, the early and late, become equal at a distinct time constant ($\tau_P = 100$ ms, Fig. 13*D*). In Fig. 13*E* ($\tau_P = 1$ s) and Fig. 13*F* ($\tau_P = 2.5$ s), respectively, only the late component occurs. Although the decay of the last curve is relatively slow, all the traces in Fig. 13 show steady state curves (interval 1 s) starting from the zero line. However, a further increase in τ_P results in an upward shift of the curve thus simulating an uncomplete retrieval of Ca^{2+} from myofilaments and the adjacent cytosol. An upward shift of the model curves may also be due to an appropriate shortening of the steady state time interval.

Discussion

As demonstrated by Kruta and Bravený (1961) the initial segment of the post-rest contraction curve (restitution of contractility) is astonishingly steep. The two-Ca store-model fails to simulate this property; this is why we have described the potentiation and staircase phenomena observed only qualitatively (Wussling and Szymanski 1973), i.e. without approximation. The modification of the model by Koester (1979, 1980) enables quantitative description of potentiation and staircase phenomena as well as interval strength relation obtained from rabbit papillary

muscles (Wussling and Szymanski 1975; Szymanski and Wussling 1978). In Koester's model, a restitutive phase during state 2 of the two Ca store model has been proposed (see Fig. 1), characterized by an enhanced equalizing flow between the stores as compared to the subsequent passive phase. Indeed, a decrease in the degree of coupling from q at nearly 1 at the beginning of the rest to $q = 0.9$ with increasing rest duration has been shown for rabbit papillary muscles (Wussling and Szymanski 1980).

The calcium movement into store 1 of the two Ca store system during state 1 is assumed to be constant. Evidently, this assumption extremely simplifies the events concerning the calcium ions entering the myocardial cell as charge carrier for the slow inward current (compare e.g. Chesnais et al. 1978; Mc Donald et al. 1980; Schulze 1981; Šimurda et al. 1981). A further simplification concerns the assumption of a complete depletion of store 2 during state 1. Even if these assumptions were modified the dynamic properties of the two Ca store system would principally not change.

Because of many structural similarities the function of the two-Ca store-model (Wussling and Szymanski 1972, 1973) may be compared with that of a two component model proposed by Edman and Johannsson (1976). Both models were primarily intended for the interpretation of contractile properties observable in rabbit papillary muscles. One difference between them is that in our model the equalizing flow between the stores is not temporarily limited, whereas in the model by Edman and Johannsson (1976) calcium which is released from compartment 2 recirculates via the contractile system and compartment 1 back to compartment 2 within a limited time, namely 0.8 second. This idea has been confirmed for the mechanical restitution of the rat papillary muscle (Ragnarsdóttir et al. 1982). Here, an additional flow from the extracellular space into the release compartment was assumed to explain the increase of the restitution curve reaching a maximum after 1—2 minutes. It seems to us that this view is more complicated than a simple interaction between coupled stores provided with leakages. From correlations of the peak rate of force development obtained from two test contractions an estimation was made of the recirculating calcium fraction which was found to vary between 0.21 and 0.72, in dependence on species as well as on preparations (Ragnarsdóttir et al. 1982; Wohlfart and Elzinga 1982). According to the model by Morad and Goldman (1973, see appendix, also compare Wohlfart 1979) the estimation of the recycling calcium fraction from contractile parameters is possible only on the basis of staircase contractions. From model calculations as well as analog simulations, as shown in the present paper, it follows that the relation between staircase phenomena and structural parameters is not unambiguous. Moreover, the amount of storage calcium released (complete or incomplete?) as well as other contributions of activator calcium are unknown. Therefore, an estimation of the recycling calcium fraction seems to be impossible on the basis of the two-Ca

store-model presented previously (Wussling and Szymanski 1972) and summarized in the present paper.

Some authors tried to predict the contraction height or the aortic pressure amplitude (man) as well as transient responses of the mammalian heart muscle by taking into consideration one (Pfeifer et al. 1980) or two (Fišer 1980) preceding time intervals in addition to other parameters. Fišer (1980) using random stimulation demonstrated in Langendorff preparations of rabbit hearts that the contraction height is determined by an interval independent term (corresponding to the transsarcolemmal Ca-flux), and by an interval dependent term (corresponding to the Ca-flux activating the contraction from the sarcoplasmic reticulum). The method of random stimulation involves relative little interval changes. Within this domain of definition, the term which depends on the preceding and prepreceding interval describes the contraction height in agreement with the two-Ca store-model.

The cooperation of Ca stores and the contractile system has been simulated on the assumption of an "expanded two-Ca store-model". Two component responses of the model might be analogous to biphasic tension development which was first demonstrated with cathodal prolongation of the action potential in calf as well as sheep ventricular strips (Kavaler 1959). Also, biphasic tension development was observed using the voltage clamp technique (see e.g. Morad and Goldman 1973). In isolated guinea pig papillary muscles two component contractions appeared after long rest periods in the presence of noradrenaline as well as dibutyryl cyclic AMP, and disappeared after the addition of verapamil (Seibel et al. 1978; Bogdanov et al. 1979). It is accepted that the early component is due to calcium which is released from an internal store, whereas the late component primarily is caused by calcium entering the cell during the action potential (Beresewicz and Reuter 1977; Lewartowski et al. 1978; Seibel et al. 1978; Bogdanov et al. 1979).

Reiter et al. (1984a, b) observed two different types of rested state contractions in guinea pig papillary muscles, an "early" one in low sodium solution and a "late" one in the presence of noradrenaline, elicited by a rather short lasting and by a rather long lasting transmembrane action potential, respectively. Using a two-compartment model they concluded that the early rested state contraction was due to calcium release from a pre-filled sarcoplasmic pool, whereas the late contraction resulted from calcium moving from the outside through the sarcoreticular network into the contractile system during the action potential. It is of interest that the "late" rested state contraction changed into a contraction with a prevailing early component (i) when the preparation was stimulated again rhythmically after the rest period, and (ii) by an increase in the steady state stimulation frequency (Seibel et al. 1978). These findings are in good agreement with corresponding model simulations although the duration of state 1 (Fig. 9) was kept constant (see Fig. 11*D* for staircase and Fig. 10 for interval dependence).

Our simulations are quite similar to Ca transients during voltage-clamp depolarizing pulses in cut skeletal muscle fibres (Schneider et al. 1981), and Ca transients during excitation-contraction coupling in the mammalian heart muscle (Allen and Kurihara 1979; Wier 1980). It should be emphasized that two components only appear in the case of two stores coupled in a distinct manner as shown with the expanded model (compare Fig. 12*B*—*F*). Supposing constant leakages and constant storage capacity, the first component disappears if the degree of coupling approaches either zero (decoupled stores) or unit (one store only). The absence of potentiation phenomena in the frog myocardium (Antoni et al. 1969; Szymanski 1985) might be due to a similar situation.

Finally, from model simulations (cf. Fig. 13) it is suggested that an effective calcium-sequestering system which is assumed to be present in the so-called "activation independent" relaxing myocardium (Brutsaert et al. 1978, 1980) is an important prerequisite for the development of two component contractions. Lecarpentier et al. (1979) showed that the relaxation of frog ventricular strips is "activation dependent" (i.e. relatively slow) due to a sparse sarcoplasmic reticulum which is less effective concerning Ca^{2+} sequestration than that of cardiac muscles of most mammalian species. Therefore it is not astonishing that biphasic tension development has been observed in several mammalian myocardial preparations (e.g. cat, calf, guinea pig) (Reuter 1974; Allen et al. 1976; Beresewicz and Reuter 1977; Reiter et al. 1984a), but never in the frog myocardium. Caffeine as well as other methylated xanthine derivatives are known to promote the release and to inhibit the uptake of calcium by vesicles derived from the sarcoplasmic reticulum of muscles (for references see Blinks et al. 1972). In heart muscle preparations caffeine induces disappearance of the first component of a biphasic contraction (Bogdanov et al. 1979) as well as of potentiation and staircase phenomena (Wussling and Szymanski 1982). These findings correspond to properties of the model mentioned above if a degree of coupling of nearly one is chosen (coupling time constant nearly zero), i.e. if the Ca store system is restricted to one store (compare Figs. 2, 6, 7, 11*C*).

The sarcoplasmic reticulum of the mammalian myocardium is considered to be one, but by no means the major, source of activator calcium for the contractile system (Mullins 1981). Measurements in cat and dog heart muscle preparations by the voltage clamp method (Šimurda et al. 1981) clearly showed that a descending staircase may be accompanied by a progressive increase as well as a decrease of the slow inward current (depending on the test pulse level), and that the relation between the contractile response and the slow inward current strongly depends on the conditions ("filled Ca pool" or "empty Ca pool") prior to the test pulse. Bers (1983) showed by measurements of tension and Ca_o depletion in rabbit papillary muscles that the Ca influx seems to be in the order required for myofilament activation with the exception of the first beat after a rest. Cumulative extracellular

Ca depletions during positive staircase after rest in guinea pig atria were observed by Hilgemann et al. (1983) using dyes which did not cross the sarcolemma. Our model simulations do not contradict these results. Even if the calcium movement into the cell is considered to be constant per beat (as a matter of fact, this assumption was made for all the simulations shown here) both Ca store models show properties which look like staircase phenomena reported recently (e.g. Bers 1983; Hilgemann et al. 1983) or previously (e.g. Woodworth 1902; Meijler 1962). Although numerous investigations (for ref. see Langer et al. 1982) have indicated that the myocardial contraction is essentially controled by Ca bound to the sarcolemma-glycocalyx, they do not rule out the control function of the sarcoplasmic reticulum the relevance of which for contraction and relaxation was emphasized of Fabiato and Fabiato (1979). Langer et al. (1983) showed in cultured and adult myocardium of rabbits by measurements of the calcium exchange as well as active and rest tension that the calcium pumping requirement might be shifted from the sarcolemma to the sarcoplasmic reticulum after application of vanadate which, in low concentrations, inhibits the SL Ca pump. It seemed therefore to be reasonable to include SL as well as SR into the model considerations.

Acknowledgement. We are grateful to our colleague Dr. G. Koester for many valuable suggestions and helpful discussion concerning the model curves of the chapter I. Our thanks are also due to Professor L. Zett for his constructive criticism of the manuscript and for his continued advice throughout the course of this work.

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Received December 5, 1984/Accepted July 15, 1985