The Isometric Twitch of Rabbit Papillary Muscle: Reflection of the Cellular Calcium Movements?*)

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Abstract. The cycling of the activator-Ca of the myocardium is mediated by the sarcolemma (SL) and the sarcoplasmic reticulum (SR). Both the extent and the time course of the release as well as of the removal of the activator-Ca by the SL differ from that by the SR. The visualization of these differences in the isometric myograms of isolated myocardium (Bogdanov et al. 1979; Günther et al. 1986; King and Bose 1983; Malecot et al. 1986) prompted the conclusion that distinct cellular Ca movements determine distinct parts of the isometric contraction-relaxation cycle. To test this hypothesis the effects of Ca, isoprenaline and ouabain on the isometric contraction-relaxation cycle of rabbit papillary muscles were re-evaluated. The similarities and differences in the effects of the interventions on the twitch measures could be explained by their effects on the cellular Ca movements.

Key words: Excitation-contraction-relaxation coupling — Rabbit papillary muscle — Isometric twitch — Cellular calcium movements

Introduction

Excitation-contraction coupling in the myocardium includes activation-contraction and inactivation-relaxation coupling (Brutsaert 1987). The transient binding of calcium ions (Ca) to troponin C appears to be an important step within both processes (Brutsaert et al. 1978). The activator-Ca which initiates activation-contraction coupling is released from both the sarcolemma (SL) and the sarcoplasmic reticulum (SR) (Bers 1985). Both structures are also primary in mediating the removal of the activator-Ca from the myofilaments and thus in inactivation-relaxation coupling (Caroni and Carafoli 1980; Chapman 1983;

*) Preliminary reports of parts of the results have already been published (Günther et al. 1986; Schubert et al. 1987).

Chapman et al. 1983; Reinlib et al. 1981). However, their Ca fluxes differ from one another in their extent and time course whithin a single excitation-contraction-relaxation cycle (Bogdanov et al. 1979; Chapman and Rodrigo 1985; King and Bose 1983; Malecot et al. 1986; Morad and Rollet 1972; Rosenstraukh et al. 1980; Roulet et al. 1979). These findings led us to hypothesize that each of the above Ca fluxes determines a distinct part of the twitch. Accordingly, selective modification of one of the Ca movements can be expected to be accompanied by simultaneous alteration of "its" twitch characteristic(s). To corroborate this hypothesis, we analyzed the isometric twitch of the rabbit papillary muscle before and during interventions which are generally accepted to have different effects on the different Ca fluxes. A set of 18 characteristics was chosen each describing either the extent or the time course of a distinct part of the twitch.

Materials and Methods

Preparation and solution. Papillary muscles of 4 to 6 mm in length and a diameter of less than 1 mm were isolated from the right ventricle of adult rabbits (3.5—4.0 kg) and mounted horizontally in a tissue bath. Using small clips of tungsten wire, one end of the preparation was connected to the wall of the experimental chamber and the other to a capacitive force transducer (DISA). The solution superfusing the muscles with a rate of 2 ml/min contained (mmol/l): NaCl 140, KCl 2.7, $CaCl_2$ 1.0, $MgCl_2$ 0.75, TRIS-HCl 10, glucose 11.1, pH 7.35 at 32 °C. It was bubbled with 100% oxygen.

Apparatus and recording. Field stimulation was delivered at 0.5 Hz between two parallel platinum plates by a square wave stimulator. The impulses of alternating polarity were of 15 ms duration and the current was 5 mA above the level that produced no further rise of maximum developed force (Fmax). This kind of stimulation and the low preload of only 3.92 mN were used to ensure both recruitment and simultaneous action of all living cells of the preparation (Blinks 1966) and to reduce their electrical and mechanical nonuniformity (Brutsaert 1987). The isometrically developed force and the electronically differentiated force signal (dF/dt) were recorded simultaneously on a light-line recorder (8 LS-1, VEB Meßgerätewerk Zwönitz, GDR).

Experimental protocol. The muscles were allowed to equilibrate in the above solution until reaching a stable level of Fmax (ca. 45 min). The twitches recorded at the end of the equilibration period served as controls. Thereafter the composition of the bathing solution was changed as follows: 1) Addition of 0.5 mol/l stock solution of $CaCl_2$ for cumulative elevation of Ca_0 to (mmol/l) 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0. At any concentration the response was recorded when the new steady state has been attained. 2) Addition of 3×10^{-5} mol/l stock solution of isoprenaline (L-isoproterenol-hydrochloride, Iso, Sigma GmbH Munich, FRG), containing 1 mg/ml ascorbic acid as anti-oxidant, for cumulative elevation of concentration to (mol/l) $3\times$ and 8×10^{-10}, $1.8\times$, $4.8\times$, an 9.8×10^{-9}, $2\times$ and 7×10^{-8}, $1.7\times$ and 3.7×10^{-7}. Twitch recordings as in 1). 3) A 3×10^{-5} mol/l stock solution of ouabain (*g*-strophantin-oktahydrate, Ou, SERVA-Feinbiochemica Heidelberg, FRG) was added to get a single concentration of 3×10^{-7} mol/l since Ou has

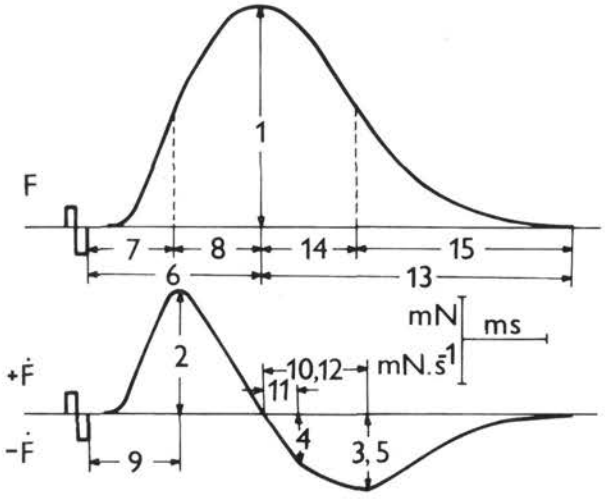

Fig. 1. The isometric twitch characteristics of rabbit papillary muscle. Force (*F, top*), d*F*/d*t* (*Ḟ, bottom; +Ḟ* and *−Ḟ* correspond to contraction and relaxation, respectively); 1 *F*max: maximum force; 2 *+Ḟ*max: maximum contraction rate; 3 *−Ḟ*max: maximum relaxation rate; 4 $-\dot{F}_1$: maximum rate of early relaxation; 5 $-\dot{F}_2$: maximum rate of late relaxation; 6 *TPF*: time to peak force*; 7 TF_{50}: time to 50% of *F*max*; 8 *TPF*-TF_{50}: time from 50% to *F*max; 9 *TP* $(+\dot{F})$: time to peak $+\dot{F}$*; 10 *TP* $(-\dot{F})$: time to peak $-F$**; 11 *T* $(-\dot{F}_1)$: time to $-F_1$**; 1 *T* $(-\dot{F}_2)$: time to $-\dot{F}_2$**; 13 *RT*: relaxation time**; 14 RT_{50}: time to half maximum relaxation. **; 15 *RT*-RT_{50}: time from 50% to full relaxation. *: measured from end of stimulus; **: measured from onset of relaxation.

a time-dependent effect with a maximum after 30 to 70 minutes. Since there may be a temperature-dependent quantitative difference in the effects of Ou in the range of 31 °C to 33 °C (Spoor et al. 1978), preparations reaching maximum response before the 50th minute after the drug application were discarded.

Statistical analysis. Arithmetic means ± standard error of the mean (SEM) are given. The significance of differences was tested by the WILCOXON-test for paired variables at P less than or equal to 0.05.

Twitch characteristics measured. The characteristics describing the contraction-relaxation cycle are shown in Figure 1. Additionally, the ratios *F*max/ + *Ḟ*max, *F*max/ − *Ḟ*max, and + *Ḟ*max/ − *F*ma were calculated and given in relative units.

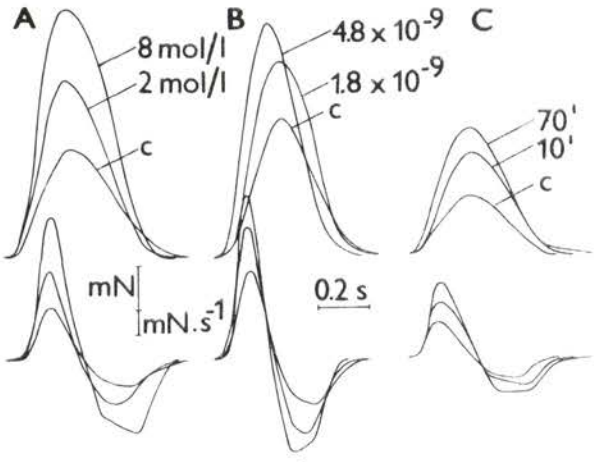

Fig. 2. Superimposed recordings of isometric twitches of rabbit papillary muscles. Upper panel: force; lower panel: dF/dt; *A*: responses to increasing Ca_o; *B*: responses to increasing Iso concentration (mol/l); *C*: time-dependent response to 3×10^{-7} mol/l Ou (minutes); note the reduction of *TPF* and *TP* ($-\dot{F}$) at 2.0 mmol/l Ca_o and 4.8×10^{-9} mol/l Iso as well as the inverse alteration of the $-\dot{F}_1/-\dot{F}_2$ relation at 8.0 mmol/l Ca_o and Ou action. *c*: control conditions, 1.0 mmol/l Ca_o without any drug. Horizontal bar: time scale. Vertical bars: *top* — force scale, *bottom* — dF/dt scale (1 mN and 10 mN/s, respectively, except 8 mmol/l Ca_o with 2mN and 20 mN/s, respectively).

Results

Elevation of Ca_o. Raising Ca_o from 1.0 to 10.0 mmol/l caused the known concentration-dependent positive inotropic effect with a rise in *F*max from 6.9 ± 1.0 mN to 39.4 ± 4.8 mN ($n = 7$). It was accompanied by a change in shape of the twitch (Fig. 2*A*). This change, however, was different at distinct Ca_o as shown by the appearance of different response patterns of the twitch characteristics. One pattern was observed between 1.5 and 3.0 mmol/l. It is characterized by alterations of measures describing the late phase (second half) of contraction and the early phase (first half) of relaxation (Table 1*a*). Above 3.0 mmol/l this pattern disappeared and another one appeared, showing selective alteration of the late phase (second half) of relaxation with all other parts of the twitch being changed proportionally to one another (Table 1*b*).

Isoprenaline. Like Ca_o, Iso had a concentration-dependent positive inotropic

Table 1. The response patterns of the isometric twitch characteristics of the rabbit papillary muscle at Ca_o (mmol/l) *a*) 1.5—3.0 (low Ca_o) and *b*) 4.0—10.0 (high Ca_o); at Iso concentrations (mol/l) *c*) 3×10^{-10}—1.8×10^{-9} (low Iso), and *d*) 4.8×10^{-9}—3.7×10^{-7} (high Iso), and *e*) 3×10^{-7} mol/l ouabain (Ou). For definitions of the twitch characteristics see legend to Figure 1. +: rise, −: decline, =: no change, each compared with control; O: not present; (): moderate.

Characteristic	*a*	*b*	*c*	*d*	*e*
*F*max	+	+	+	+	+
$+\dot{F}$max	+	+	+	+	+
*F*max/$+\dot{F}$max	−	=	=	−	=
$-\dot{F}$max	+	+	+	+	+
*F*max/$-\dot{F}$max	−	=	=	−	+
$+\dot{F}$max/$-\dot{F}$max	≈	=	=	=	+
TPF	−	=	=	−	=
TF_{50}	≈	=	=	−	=
$TPF\text{-}TF_{50}$	−	=	=	−	=
$TP\ (+\dot{F})$	≈	=	=	−	=
$TP\ (-\dot{F})$	−	=	−	−	=
RT	(−)	−	=	−	+
RT_{50}	−	=	=	−	=
$RT\text{-}RT_{50}$	≈	−	=	(−)	+
$-\dot{F}_1$	+	+	+	+	+
$-\dot{F}_2$	+	+	+	+	+
$T\ (-\dot{F}_1)$	≈	(+)	=	=	=
$T\ (-\dot{F}_2)$	≈	+	=	=	=
relation of $-\dot{F}_1$ to $-\dot{F}_2$	0	≪	<	>	</=

Table 2. Inotropic state of rabbit papillary muscles. The means ± standard error of the mean (SEM) ($n = 7$) of the maximum rate of rise of force, $+\dot{F}$max (mN/s) are given, at Ca_o (mmol/l) *a*) 3.0, and *b*) 8.0; at Iso concentrations (mol/l) *c*) 1.8×10^{-9}, *d*) 7×10^{-8}, *e*) 3.7×10^{-7}; and *f*) 3×10^{-7} mol/l ouabain (70 minutes after the drug application). Similar response patterns of the twitch characteristics occurred at i) *a* and *d*, ii) *c* and *f*, and iii) *b* and *e*.

	a	*b*	*c*	*d*	*e*	*f*
Mean	111.7	224.0	52.4	120.7	192.4	73.7
SEM	14.7	26.9	9.4	23.6	47.1	11.2

effect. It elevated *F*max from 6.8 ± 1.2 mN (control) to 23.8 ± 5.9 mN ($n = 7$) at 3.7×10^{-7} mol/l. Again, different response patterns of the twitch characteristics were observed at different Iso concentrations (Fig. 2*B*). The first one

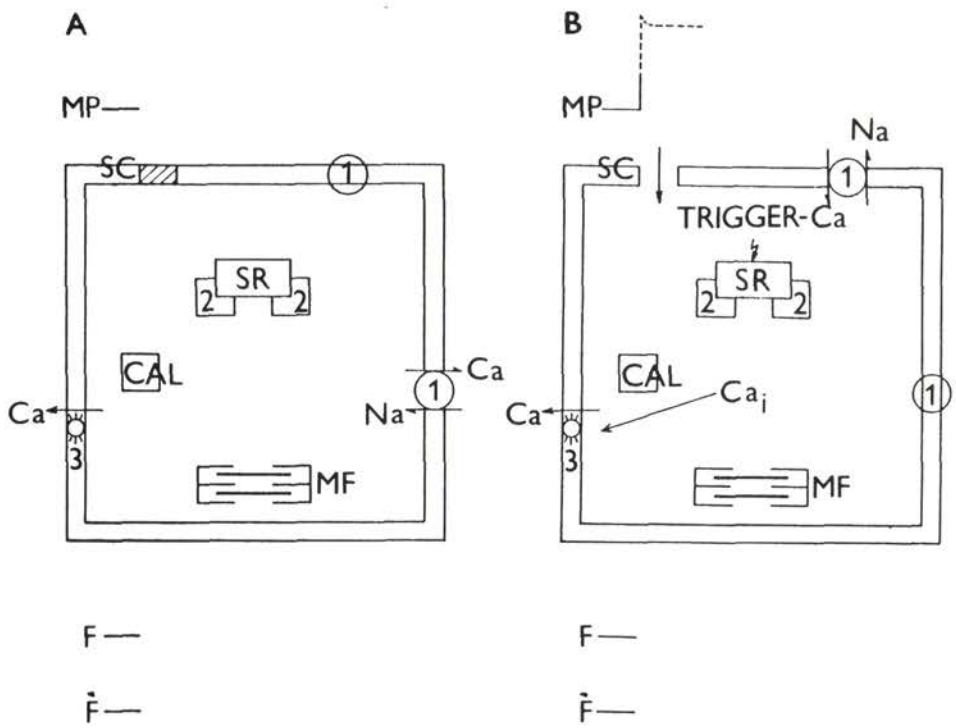

Fig. 3. Possible connection between cellular Ca^{2+} movements and shape of the isometric twitch during diastole (*A*) and excitation (*B*). MP: membrane potential; *F*: force; $\dot{F}$: dF/dt. The squares represent the myocardial cell. SC: slow channel; Na: sodium; Ca: calcium; Ca_i: intracellular free Ca concentration; SR: sarcoplasmic reticulum; CAL: calmodulin; MF: myofibrils; 1: Na/Ca exchanger; 2: phospholamban; 3: Ca pump of SL. The arrows and their widths show the Ca movements and their relative contribution to excitation-contraction-relaxation coupling of the rabbit myocardium. The broken lines indicate the new situation. A detailed explanation is given in the text.

appeared at low Iso levels, i.e. between 3×10^{-10} and 1.8×10^{-9} mol/l (Table 1*c*). Like the pattern at high Ca_o it is characterized by proportional changes of all parts of the twitch, except the reduction of the late relaxation which is evident at high Ca_o but not at low Iso levels. Both patterns differ from one another in another aspect: The shortening of *TP* ($-\dot{F}$) occurred at low Iso (as at low Ca_o) but not at high Ca_o.

A second pattern was found above 1.8×10^{-9} mol/l (Table 1*d*). It is qualitatively similar to that at low Ca_o but quantitatively more pronounced. Moreover, the reduction of TF_{50} is involved in *TPF* shortening in addition to that of

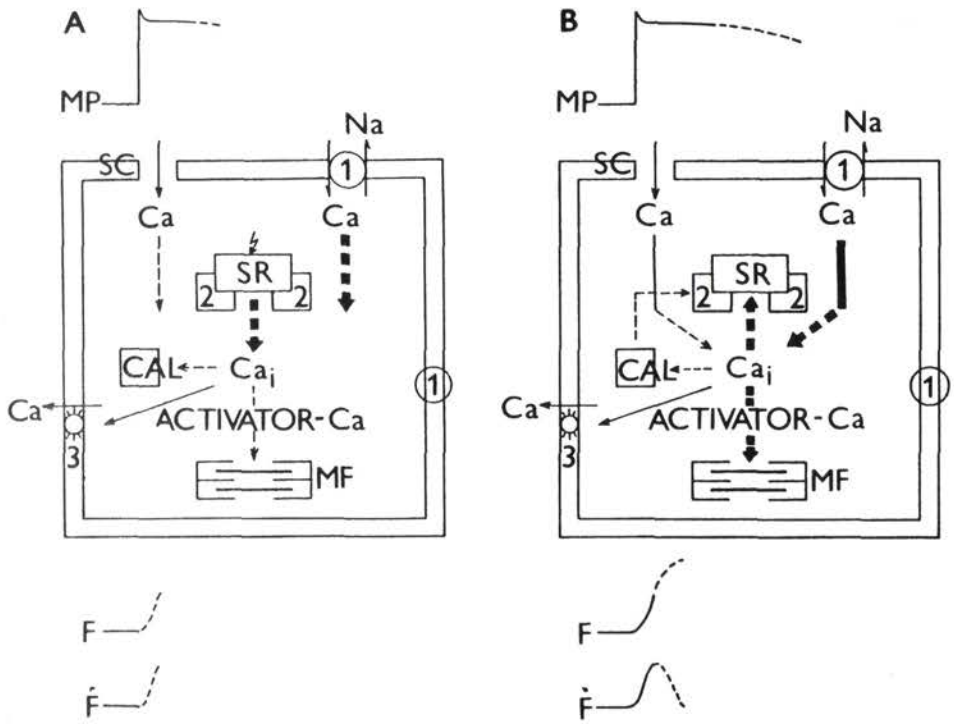

Fig. 4. Possible connection between cellular Ca^{2+} movements and shape of the isometric twitch during early (*A*) and late force development (*B*). Definitions for abbreviations and numbers are the same as in Figure 3. A detailed explanation is given in the text.

TPF-TF$_{50}$, but only above 2×10^{-8} mol/l. The shortening was observed up to 3.7×10^{-7} mol/l. In contrast, *TPF-TF*$_{50}$ continued shortening only up to 1.7×10^{-7} mol/l. At the highest concentration it returned towards the control level. Furthermore, the decline of *RT* was found to be mainly due to that of the early phase, *RT*$_{50}$, whereas *RT-RT*$_{50}$ was altered only moderately. This change was accompanied by a further reduction of *TP* ($-\dot{F}$) which, in turn, was due to the shift of $-\dot{F}$max from $-\dot{F}_2$ to $-\dot{F}_1$ whithout a change in $T(-\dot{F}_1)$ or $T(-F_2$ (Table 1*d*).

Ouabain. Ou showed a time-dependent positive inotropic effect. *F*max reached its highest level, 11.5 ± 1.8 mN ($n = 7$), 60 minutes after the addition of the drug. The rise in force was accompanied by the appearance of a response pattern of the twitch characteristics (Table 1*e*, Fig. 2*C*) that was distinctly marked by the interval of drug action. In most parts this pattern was identical with those

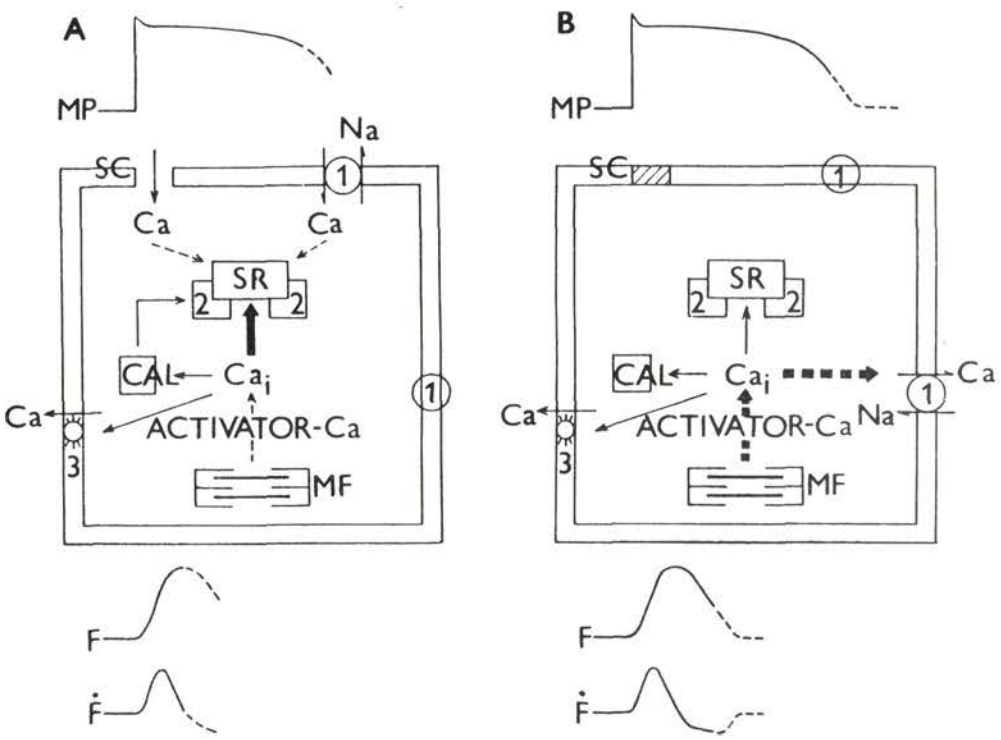

Fig. 5. Possible connection between cellular Ca^{2+} movements and shape of the isometric twitch during early (*A*) and late relaxation (*B*). Definitions for abbreviations and numbers are the same as in Figure 3. A detailed explanation is given in the text.

found at high Ca_o and low Iso with the following exceptions: the proportional changes of the early and late contraction and early relaxation were parallelled by a selective prolongation of the late relaxation. Simultaneously, $-\dot{F}$max, reached in $-\dot{F}_2$, grew less then $-\dot{F}_1$ and also less than Fmax and $+\dot{F}$max, the latter leading to an increase in the ratios Fmax/$-\dot{F}$max and $+\dot{F}$max/$-\dot{F}$max (Table 1).

Finally, the patterns which resembled one another appeared at comparable inotropic states, as indicated by the levels of $+\dot{F}$max, independent of the kind of intervention (Table 2).

Discussion

We hypothesized that distinct parts of the isometric contraction-relaxation curve of isolated myocardial preparations under the experimental conditions used in the present study are determined by distinct cellular Ca movements involved in activation and inactivation of contraction. Thus, the alteration of any one of them can be expected to become reflected in the concomitant alteration of "its" twitch characteristic(s), i.e., of those parameters which are determined by the affected Ca flux. This hypothesis is based on the observation that Ca released from the SR determines the early and that delivered by the SL the late phase of contraction as demonstrated by the two-component contraction (Bogdanov et al. 1979; King and Bose 1983; Malecot et al. 1986; Rosenstraukh et al. 1980). There are also two consecutive phases of relaxation. The later one has been shown to be related to Ca extrusion from the myoplasm via the sarcolemmal Na-Ca exchange carrier (Chapman and Rodrigo 1985; Roulet et al. 1979). Also, it is theoretically likely that the Ca movements mediated by the latter transport system occur later during the contraction-relaxation cycle than those mediated by the Ca pump of the SR, which is the second Ca extrusion mechanism able to bring about relaxation of the single twitch (Caroni and Carafoli 1980; Chapman 1983; Chapman et al. 1983; Reinlib et al. 1981). The direction of the carrier-mediated Ca transport depends on the membrane potential: Ca enters the cell during depolarisation and leaves it at potentials negative to the "reversal potential" of the exchanger (Mullins 1984). Because this potential might be a negative one (Chapman 1983; Mullins 1984) the action potential (AP) plateau that is rather long in the rabbit papillary muscle (Johannsson and Wohlfart 1980) must pass before Ca extrusion is initiated. In contrast, the Ca uptake into the SR might rather interfere with the early relaxation. This is suggested by the aequorin signal which is supposed to reflect those parts of the intracellular Ca transient which are induced by the Ca movements across the SR membrane (McKinnon and Morgan 1986; Morgan et al. 1984). The decay phase of the aequorin signal is determined by the reuptake into the SR of the released Ca (Fabiato 1985 Morgan et al. 1984). It begins well before the end of the AP plateau.

Accordingly, alteration of Ca release from or reuptake into the SR is expected to be accompanied by changes of those characteristics which describe the early phase of contraction and relaxation, respectively. Influences on activator-Ca movements mediated by sarcolemmal systems, on the other hand, are expected to be parallelled by changes of the late contraction and late relaxation characteristics.

To test this assumption, isometrically contracting rabbit papillary muscles were exposed to either different Ca_o, Iso or Ou. The mechanograms recorded

under these conditions were analysed by a set of different parameters each describing either the extent or the time course of a distinct part of the twitch (Fig. 1). To describe the early and the late phases of contraction and relaxation, both the time to peak force, *TPF*, and the relaxation time, *RT*, have been devided into two intervals thus getting rough but simply evaluatable measures for the respective phases.

Raising Ca_o elevates the transsarcolemmal Ca influx during excitation via both, the slow channel (Beeler and Reuter 1977) and the Na-Ca exchanger (Mullins 1984). This increases the amount of SL-derived Ca (SL-Ca) supplying the activator-Ca pool (Bers 1985). Additionally, it augments Ca release from the SR due to its graded trigger effect (Fabiato and Fabiato 1978) thereby elevating the amount of SR-derived Ca (SR-Ca) within the activator-Ca pool as shown by the aequorin signal (Morgan et al. 1984). The increased activator-Ca concentration, in turn, elevates the extent and the rate of force development due to the increase in the number of contracting sites and in the rate of their activation (Adler et al. 1979). The Ca extrusion mechanisms, the Ca pump of the SR, and the sarcolemmal Na-Ca exchange carrier are stimulated by the Ca concentration in their vicinity like the release mechanisms. Thus, their activity also increases (Kimura et al. 1986; Tada and Inui 1983). Thus, the early and the late removal of activator-Ca from the myofilament space, i.e., the early and the late relaxation, are stimulated by a common event, Ca itself, as are both phases of contraction. The resulting proportional stimulation of the Ca movements involved in activation and inactivation of contraction might cause the proportional alteration of the contraction-relaxation cycle which is usually reported to occur under conditions which elevate the Ca availability for the contractile apparatus (Morgan et al. 1984; Scholz 1980; Skomedal et al. 1982; 1987). However, the rise in the intracellular Ca concentration (Ca_i) also increases the Ca binding to calmodulin. The Ca-calmodulin complex (Ca-Cal), in turn, activates a Ca-Cal-dependent protein kinase that predominantly phosphorylates phospholamban, the Ca pump regulator protein of the SR, thereby enhancing the Ca uptake into the SR. If this stimulation acts in addition to that mediated by Ca itself, the cellular Ca regulation will be influenced asymmetrically as will be its reflection, the isometric twitch characteristics. This was indeed observed by the selective shortening and acceleration of the late contraction and early relaxation at Ca_o between 1.5 and 3.0 mmol/l. The following findings support these "low-Ca_o" effects to be the reflection of the selective Ca-Cal-dependent stimulation of the SR Ca uptake superimposed on the proportional Ca-dependent activation of contraction and relaxation:

1) The response pattern observed at low Ca_o is very similar to that at high Iso, suggesting similar effects on the cellular Ca regulation under both con-

ditions. The response pattern induced by Iso, the "β-type response" (Aass et al. 1983; Scholz 1980; Skomedal et al. 1982), is generally assumed to be related to the selective net stimulation of the Ca uptake by the SR (see below). The rise in Ca_o or Ca_i, however, does not activate the cAMP-system (Dönges et al. 1977) which is generally supposed to be responsible for this kind of response, but stimulates the SR Ca pump via Ca-Cal in vitro (Kirchberger and Antonetz 1982; Tada and Inui 1983; Tada et al. 1983).

2) These Ca-Cal-dependent effects on the SR Ca uptake in vitro are limited to an optimum Ca concentration that is relatively low (Kirchberger and Antonetz 1982) with respect to the range within which activation of contraction from threshold at 10^{-7} mol/l to maximum at 10^{-5} mol/l is observed (Fabiato and Fabiato 1978; Marban et al. 1980). The same has been assumed to be valid for the rat heart (Vittone et al. 1985). The present results show that the response pattern in question is also limited to a distinct Ca concentration that is a relatively low one with respect to 10.0 mmol/l which, in turn, is still submaximum for force development in rabbit papillary muscle. Below and above this concentration range the response pattern disappeared as did the Ca-Cal-dependent Ca pump activation (Kirchberger and Antonetz 1982).

3) Lindemann and Watanabe (1985) found neither elevated phosphate incorporation into phospholamban nor stimulation of Ca-Mg-ATPase activity of the SR during the rise in Ca_o. However, their experiments on rat and on guinea-pig myocardium started at Ca levels as high as those at which Vittone et al. (1985) assumed that process to be already saturated and at which in the present study the response pattern disappeared. This suggests that the Ca concentrations used by Lindemann and Watanabe (1985) were already supramaximum for the Ca-Cal-dependent stimulation of the SR Ca uptake. This is supported by our results showing the disappearance of the response pattern above 3.0 mmol/l Ca_o (see also below).

4) The selective net stimulation of the Ca uptake by the SR might selectively favor the relaxation and, due to that, inhibit the force development. Again, this is what we observed: the shortening of RT, indicating enhanced relaxation, and the reduction of TPF and the decline of the $F\text{max}/-\dot{F}\text{max}$ ratio, indicating inhibited force development. The fact that the shortening of RT is mainly due to that of RT_{50} and that there is a simultaneous enhancement of the early relaxation rate as shown by the shift of $-\dot{F}\text{max}$ from $-\dot{F}_2$ towards $-\dot{F}_1$ supports the idea that alterations of the Ca uptake by the SR are reflected in those characteristics which describe early relaxation. Furthermore, we showed that the shortening of TPF is exclusively due to that of the late phase of contraction (TPF-TF_{50}). This again agrees with our hypothesis that the characteristics of the late contraction reflect the balance between transsarcolemmal Ca influx and Ca uptake by the SR. The latter starts just after the release from the

SR has stopped (Fabiato 1985) while the supply of SL-Ca to the activator-Ca pool still continues. Under these conditions, the Ca pump of the SR and the contractile apparatus compete for the SL-Ca. Thus, the more the pump will be activated the more of the SL-Ca, which determines the late phase of contraction (Bogdanov et al. 1979; King and Bose 1983; Malecot et al. 1986; Rosenstraukh et al. 1980), will be prevented from contraction activation thereby selectively shortening the late contraction and diminishing the extent of force development. As suggested above, the rise in Ca_o and Ca_i beyond the optimum range for Ca-Cal-dependent mechanisms should result in a Ca-Cal decrease accompanied by the disappearance of the twitch alterations induced by them; at the same time, the effects of Ca itself become unmasked again. The present results agree with this idea as shown by the disappearance of the response pattern seen at low Ca_o and the appearance of proportional changes of most parts of the twitch at Ca_o above 3.0 mmol/l. However, at these high Ca_o another alteration of the twitch is evident: a shortening of *RT* that is exclusively due to the reduced duration of the late relaxation (RT-RT_{50}) and is accompanied by the return of $-\dot{F}_{max}$ from $-\dot{F}_1$ to $-\dot{F}_2$. The late relaxation has been shown to be easily influenced by interventions which interfere with the sarcolemmal Na-Ca exchange mechanism (Chapman and Rodrigo 1985; Roulet et al. 1979). In the light of this, the response pattern observed at high Ca_o may be interpreted reflecting the selective stimulation of Ca extrusion by the Na-Ca exchange. This assumption is supported by the following: 1) The rise in Ca_i increases the membrane current carried by this system (Kimura et al. 1986). 2) The rise in Ca_o is parallelled by the shortening of the AP duration (Johannsson and Wohlfart 1980). Thus, the reversal potential of the exchange will be reached earlier. The result may be that the outwardly directed action of the carrier will be initiated earlier thereby reducing the time the carrier needs to accomplish the necessary Ca extrusion. 3) Potentiated twitches as those occurring after an interpolated extra stimulus or following a transient increase of the pacing frequency are induced by shorter APs than the rhythmically induced ones (Wohlfart and Elzinga 1982). These twitches, like those at high Ca_o, show shortened late relaxation and a stronger rise of $-\dot{F}_2$ than of $-\dot{F}_1$ (own unpublished results). The "extra-Ca" which is responsible for the twitch potentiation appears measurable in the extracellular fluid within the same twitch (Hilgemann and Langer 1984). This again indicates an augmented transsarcolemmal Ca efflux.

Iso is known to act via activation of the sarcolemmal β-receptor coupled adenylate cyclase. The subsequent increase in the myoplasmic cAMP concentration activates a cAMP-dependent protein kinase that phosphorylates several cellular proteins. The main targets for the kinase's action important for excitation-contraction-relaxation coupling are the sarcolemmal slow Ca channel,

phospholamban, and the Na-Ca exchange, in addition to the inhibitory subunit of troponin (TnI) (Katz 1983). Comparing these target structures with those of the elevated Ca_o, there is no considerable difference, except TnI. Thus, if our hypothesis is correct, in the case of Iso action one has to expect alterations of the processes of cellular Ca handling and of the twitch characteristics similar to those at elevated Ca_o; there is no hint to assume that the stimulation of the Ca extrusion mechanisms is stronger than that of the release mechanisms. The main deviation from the "poorly" Ca mediated alteration of cellular Ca regulation is that during Iso action the activation of the above processes occurs twice, i) via the cAMP system, and ii) additionally and secondary to the former, via Ca itself. The present results show that rising Iso concentration induces response patterns of the twitch characteristics similar to those of raising Ca_o, i.e., i) a proportional alteration of contraction and relaxation at low Iso levels, ii) the typical "β-type response" (Scholz 1980; Skomedal et al. 1982; 1987), that is the selective promotion of relaxation, at intermediate Iso concentrations, and iii) a tendency of the response to return from the second to the first pattern when the highest concentration is used. Besides giving support to our hypothesis these results suggest that the "β-type response" is not directly due to the cAMP-dependent stimulation of the relaxing processes but to the Ca-Cal-dependent one. Although no direct evidence of this assumption can be provided by the present study, there are some hints supporting it: 1) The similarity of the response patterns induced by either raising Ca_o or Iso may indicate a similar alteration of the underlying cellular processes under both conditions. 2) The appearance of either of these response types depends more on the inotropic state of the preparation than on the intervention. This is suggested by the levels of $+\dot{F}max$. They are directly proportional to Ca_i (Yue 1987), and of comparable extent under the conditions which produce comparable response patterns (Table 2). 3) The activity of the Ca-Cal-dependent system is evident during β-receptor stimulation (Aass et al. 1983; Lindemann and Watanabe 1985), it needs a certain Ca level to be active, and the qualitative measures of relaxation are more susceptible to inhibition than those of contraction (Aass et al. 1983).

In the framework of our hypothesis, the twitch alterations induced by Iso can be explained as follows: The cAMP-dependent phosphorylation of the slow Ca channel increases the transsarcolemmal Ca influx (Bean et al. 1984; Brum et al. 1984). This augments the Ca release from the SR (Fabiato 1985, Fabiato and Fabiato 1978). The cAMP-dependent phospholamban phosphorylation (cAMP-PP) increases the Ca reuptake by the SR which i) compensates for the enhanced transsarcolemmal Ca influx due to the uptake of some of the SL-Ca before it reaches the myofilament space, and ii) adds to the increased release from the SR due to its increased filling level (Fabiato 1985; Fabiato and Fabiato 1978). These processes may be the origin of the positive inotropic effect of Iso

which is accompanied by a proportional change of the twitch characteristics. The shortening of TP ($-\dot{F}$) which, in contrast to the response pattern at low Ca_o, parallels these alterations, may be due to the lower Ca sensitivity of the phosphorylated troponin (Katz 1983) and/or to the elevated Ca uptake by the SR. The latter is caused by a double stimulation of the SR Ca pump by i) Ca itself, and ii) cAMP-PP during Iso action, but solely by the Ca mediated one at low Ca_o (Tada and Inui 1983). The situation only changes when Ca_i has reached its optimum for Ca-Cal-dependent phospholamban phosphorylation (Ca-Cal-PP). In this case, the additional stimulation of the Ca pump of the SR may be responsible for the so called "β-type response" (Scholz 1980; Skomedal et al. 1982), including the alterations of the twitch which have been reported for the first time in the present paper. They are evident at the intermediate Iso concentrations. As Ca_i rises above its optimum for this stimulation, the latter response pattern will disappear and a proportional change of the contraction-relaxation cycle will reappear. This is indicated by the present results at the highest Iso concentration used.

Ou is known to exert its influence on the cellular Ca regulation by interfering with the sarcolemmal Na-Ca exchange mechanism. Nevertheless, it is not quite clear whether this interference results primarily from the increase in the internal Na concentration due to inhibition of the Na-K-ATPase and subsequent competition between Na and Ca at the internal binding sites of the carrier (Barry et al. 1985; Huang et al. 1979; Poole-Wilson et al. 1979; Reeves and Sutko 1983), or from the change of the membrane potential induced by the glycoside (Boyett et al. 1986). Whichever the real mechanism is, the final pathway might be the same in both cases and it may involve the enhancement of Ca entry during depolarisation and inhibition of outlet at membrane potentials negative to the reversal potential of the Ca exchange via the SL (Mullins 1984). The consequences of the increased Ca influx may again be the same as already discussed: only one stimulus, Ca itself, activates the processes of contraction-relaxation coupling. This may result in their proportional activation and thus in a proportional alteration of the twitch characteristics. The present results show that this is the case for the measures which describe the early and the late phase of contraction and the early phase of relaxation. The late phase of relaxation, however, is selectively prolonged, and its rate is less augmented than Fmax, $+\dot{F}$max, and $-\dot{F}_1$. This is again in agreement with our hypothesis. As stated above, the participation in the relaxation of Ca extrusion via Na—Ca exchange may occur later than that of the Ca uptake by the SR. Thus, its alteration can be expected to be accompanied by alterations of the characteristics of the late relaxation. In contrast to the stimulation of the outwardly directed carrier-mediated Ca transport, proposed to be caused by high Ca_o, Ou has inhibitory

action. An opposite behaviour of the late relaxation characteristics for Ou and high Ca_o is also revealed by the present results.

Although Ca_i increases during Ou action and enhanced Ca binding to calmodulin is therefore to be assumed, there are no hints of a selective alteration of the early relaxation characteristics that is predicted by our hypothesis in the case of selective stimulation of the Ca uptake into the SR. This could be because i) the predicted changes of the twitch are not necessarily induced by any alteration in cellular Ca movement, ii) the predicted alteration of the twitch characteristics are compensated for by others, and/or iii) the cellular Ca movement in question is not affected by the intervention. Although the present study provides no direct evidence of Ca-Cal-PP being responsible for the alteration of the early phase of relaxation, this idea is supported by the levels of $+\dot{F}max$ observed under the influence of Ou. They are comparable with those observed with low Iso concentrations, and smaller than those at low Ca_o and high Iso (Table 2). This suggests that during Ou action Ca_i does not reach the optimum for Ca-Cal-dependent activation of the Ca pump of the SR. The last of the above explanations of the lack of changes in the early relaxation may be the most probable one.

In the light of the effects of these interventions on the cellular Ca movements in the myocardium, the observed effects of Ca_o, Iso, and Ou on the twitch characteristics of rabbit papillary muscles support our hypothesis that distinct cellular Ca movements determine distinct twitch characteristics. This hypothesis is schematically summarized in Figures 3 to 5. The low resting Ca_i is maintained by the Ca pump of the SL and by the outwardly directed Na—Ca exchange (Caroni and Carafoli 1980; Chapman 1983) (Fig. 3*A*). During the action potential (Fig. 3*B*) the transsarcolemmal Ca influx triggers the release of Ca from the SR. The Ca released from the SR (Fig. 4*A*) suddenly increases Ca_i in the vicinity of the myofilaments. Among other structures, the Ca pump of the SR, calmodulin, and troponin C (TnC) compete with each other for this Ca. The Ca that binds to TnC is called the activator-Ca. The SR-derived activator-Ca initiates the contraction. Somewhat later, possibly due to the greater diffusion distance, the SL-derived Ca adds to the activator-Ca pool (Fig. 4*B*) and determines the late phase of contraction. Following the release, the reuptake of Ca into the SR by its Ca pump interferes with a further supply of Ca from sarcolemmal sites to the activator-Ca pool. Thus, the pump activity is the second determinant of the late phase of contraction. Moreover, the Ca-pump of the SR initiates the relaxation as soon as its Ca transport exceeds the influx (Fig. 5*A*) and determines it until the membrane potential reaches the reversal potential of the Na-Ca exchange. At this potential (Fig. 5*B*) the carrier reverses the direction of its Ca transport and thus participates in removing the remaining Ca from TnC, i.e., it determines the late phase of relaxation.

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