MINIREVIEW

Regulation of Cardiac Muscle Contraction by Intracellular Ca^{2+}

Satoshi KURIHARA

*Department of Physiology, The Jikei University School of Medicine,
Minato-ku, Tokyo, 105 Japan*

Contraction of cardiac muscle is regulated by the change in intracellular free Ca concentration ($[\text{Ca}^{2+}]_i$) [1]. The steady state relation between $[\text{Ca}^{2+}]_i$ and tension can be demonstrated by measurement and analysis of the pCa-tension relation in skinned preparations [2]. However, the cardiac muscle contraction under physiological conditions is transient and measurement of the intracellular Ca^{2+} concentration change (Ca^{2+} transient) is necessary to elucidate the regulatory mechanism of contraction by intracellular Ca^{2+} . Various types of intracellular Ca^{2+} indicators are now available for the measurement of intracellular Ca^{2+} transients [3]. The measurement of $[\text{Ca}^{2+}]_i$ using an indicator with a high-time resolution has demonstrated that $[\text{Ca}^{2+}]_i$ transiently increases preceding contraction in a single twitch; this finding provides various insights into the regulatory mechanism of cardiac muscle contraction by Ca^{2+} .

Many steps related to contraction and relaxation of mammalian cardiac muscle are triggered by an increase in $[\text{Ca}^{2+}]_i$ and are involved sequentially from an increase in $[\text{Ca}^{2+}]_i$ to contraction (tension or shortening). The Ca^{2+} transient is one of the important steps which controls contraction. However, the measured intracellular Ca^{2+} is a small portion of the total Ca^{2+} which is delivered to the myoplasm following excitation of the surface membrane, because intracellular Ca^{2+} binding proteins (for example, troponin, calmodulin) bind most of the Ca^{2+} delivered [4, 5]. Therefore, the change in the affinity for Ca^{2+} of these Ca^{2+} binding proteins, particularly troponin-C, substantially influences intracellular Ca^{2+} transients.

In the present short review, factors which influence Ca^{2+} transients and contraction are summarized. Based on pertinent observations, the recent view of the regulatory mechanisms of intracellular Ca^{2+} on cardiac muscle contraction is discussed.

GENERAL SCHEME

Blinks [6] divided the processes related to cardiac muscle contraction into three main steps (Upstream, Central, and Downstream), which are useful for

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Fig. 1. General scheme showing the steps related to contraction in mammalian cardiac muscle. See text for details.

discussion on the molecular mechanism of cardiac muscle contraction (Fig. 1). "Upstream" is a step mainly related to the regulation of $[Ca^{2+}]_i$; an influx of Ca^{2+} , Ca^{2+} release, Ca^{2+} uptake, and Ca^{2+} extrusion through the cell membrane are involved. "Central" is the step of Ca^{2+} binding to troponin-C. Many factors are related to the change in the affinity of troponin-C for Ca^{2+} , which secondarily alters the magnitude and the time course of Ca^{2+} transients and contraction. "Downstream" is the process after Ca^{2+} is bound to troponin-C, which removes the inhibitory effect of troponin-I on the actin-myosin interaction [1]. Then, the cross-bridges attach thin filaments and contraction is initiated. In the present review, this general scheme was employed to classify the factors which influence the molecular mechanisms in the regulation of contraction.

MEASUREMENT OF INTRACELLULAR Ca^{2+} CONCENTRATION

Many Ca^{2+} indicators are now available for the measurement of intracellular Ca^{2+} transients. These indicators are classified into three categories: Ca^{2+} -sensitive photoproteins, fluorescent dyes, and metallochromic dyes [3]. The measurement of absorbance using the metallochromic dyes is not easy and is not practical in cardiac muscles, because the absorbance signal in cardiac muscle is small and movement artifacts seriously interfere with the Ca^{2+} signals. The first two indicators, particularly the fluorescent Ca^{2+} indicators, are now widely applied to cardiac muscles; single cells and tissues [7-9].

One of the advantages of the aequorin method which we have been employing in our studies is that the Ca^{2+} signal of aequorin is not greatly influenced by the movement of the preparations during contraction [3]. This is a great advantage over other indicators in the measurement of Ca^{2+} signals in response to mechanical perturbations. However, the aequorin light signal represents the $[\text{Ca}^{2+}]_i$ change in the superficial cells of the preparation and tension is measured from the whole preparation. Therefore, it is necessary to assume that the Ca^{2+} signals of the superficial cells are representative of all the cells in the preparation.

Since the application of aequorin to a single myocyte is difficult, many types of fluorescent Ca^{2+} indicators are now applied to single myocytes and cell length shortening is simultaneously measured (for example, Spurgeon *et al.* [8]). This method is beneficial to characterize the relation between contraction and the change in $[\text{Ca}^{2+}]_i$ in single cells. However, there are some uncertainties in the quantitative measurement of Ca^{2+} transients using fluorescent Ca^{2+} indicators [10, 11]. This is probably due to the changes in the characteristics of the indicators when injected into the myoplasm such as, compartmentalization of the indicators, binding to proteins, or the influences of intracellular ionic environment [3, 12]. In addition, the Ca^{2+} transients measured in cell length shortening are not identical to the Ca^{2+} transients measured in tension, which will be discussed later in the Downstream section.

GENERAL CONSIDERATION

The record of simultaneously measured action potential, aequorin Ca^{2+} signal and tension demonstrates that $[\text{Ca}^{2+}]_i$ rises just after the beginning of the action potential and then contraction follows, indicating that the early phase of the action potential triggers an increase in $[\text{Ca}^{2+}]_i$ (Fig. 2). Since the duration of the action potential is much longer than that of Ca^{2+} transients, the time course of the Ca^{2+} transient, particularly the decay of the Ca^{2+} transient, is not seriously influenced by membrane repolarization. However, a slight change in the decay of the Ca^{2+} transient is observed when the duration of the action potential is altered by the change in stimulation frequency.

When $[\text{Ca}^{2+}]_i$ increases, Ca^{2+} is bound to the regulatory site of troponin-C [4]. The time course of the troponin-Ca complex may be calculated using the fixed on- and off-rate constants of troponin-C for Ca^{2+} [13] (the off-rate constant might be altered in a tension-dependent manner, which will be discussed later). The troponin-Ca complex was just behind Ca^{2+} transient and preceded tension development, suggesting that the troponin-Ca complex is an intermediate step between the Ca^{2+} transient and tension (Fig. 3). The concentration of the regulatory site of troponin (low affinity site) is reported to be about $70 \mu\text{M}$; two high affinity sites are considered to be occupied by Ca^{2+} and Mg^{2+} depending on the estimated concentration of both ions in the resting state before excitation [4, 13]. In addition to troponin, calmodulin at about $50 \mu\text{M}$ is another Ca^{2+} binding protein, which

Fig. 2. Simultaneously measured records of action potential, aequorin light signal, and tension in a ferret papillary muscle. Stimulation frequency was 1/5 s. Normal Tyrode's solution at 30°C. Sixty-four signals were averaged (Tanaka and Kurihara, unpublished data). Aequorin light signal was expressed as fractional luminescence (L_f).

Fig. 3. The time courses of the intracellular Ca^{2+} transient (Ca^{2+}), troponin-Ca complex (TnCa) in a low affinity site and tension. The time course of troponin-Ca complex was calculated using the measured intracellular Ca^{2+} concentration change, which was obtained by conversion of the aequorin light signal to $[\text{Ca}^{2+}]_i$. On-rate constant (K_{on}) is $3.9 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. Off-rate constant (K_{off}) is 32.5 s^{-1} . On-rate constant is diffusion-limited [13]; the off-rate constant was calculated using the binding constant of troponin-C in the myofibril ($1.2 \times 10^6 \text{ M}$) [77]. Resting $[\text{Ca}^{2+}]_i$ was assumed to be $0.1 \mu\text{M}$ (Konishi and Kurihara, unpublished data).

regulates enzymatic activities through Ca^{2+} binding [5]. Recent results measured with various types of Ca^{2+} indicators indicate that the peak of intracellular Ca^{2+} transient in twitch is 0.5–1.5 μM [14–16] and the total released Ca^{2+} is about 130 μM [17]. Therefore, almost all Ca^{2+} increased in the myoplasm is bound to Ca^{2+} binding proteins, and intracellular free Ca^{2+} is a small portion of the total Ca^{2+} increase in the myoplasm. If the affinity of troponin-C for Ca^{2+} is increased, more Ca^{2+} is bound to troponin-C and the peak of Ca^{2+} transient is decreased accompanying the shortening of the decay time of the Ca^{2+} transient.

UPSTREAM

1. *Effects of $[\text{Ca}^{2+}]_o$*

The time course of the intracellular Ca^{2+} transient in cardiac muscle has been considered not to be influenced by $[\text{Ca}^{2+}]_o$ [14, 18]; a higher concentration of $[\text{Ca}^{2+}]_o$ increases the peak of Ca^{2+} transient and tension without changing the time courses and the opposite is true of low $[\text{Ca}^{2+}]_o$. Therefore, the relation between the peaks of Ca^{2+} transients and tension in twitch contraction is frequently used as a control in the assessment of the change in the Ca^{2+} sensitivity of the contractile elements [18]. However, when the time courses of Ca^{2+} transients and tension are carefully measured, the time courses of both signals at different $[\text{Ca}^{2+}]_o$ are not identical (Fig. 4). At a higher $[\text{Ca}^{2+}]_o$, the decay time of the Ca^{2+} signal is shortened and the relaxation time is prolonged. In addition, the time to the peak of the Ca^{2+} transient is slightly shortened at higher $[\text{Ca}^{2+}]_o$. The opposite changes are recognized at lower $[\text{Ca}^{2+}]_o$. We interpret this change in the time course of Ca^{2+} transients at different $[\text{Ca}^{2+}]_o$ to partly reflect the tension-dependent change of the affinity of troponin-C for Ca^{2+} (see later discussion in Downstream).

2. *Factors influencing Ca^{2+} release and Ca^{2+} uptake of sarcoplasmic reticulum*

1) *Factors influencing Ca^{2+} release channels in sarcoplasmic reticulum (s.r.).*

Ryanodine can specifically bind Ca^{2+} release channels of the s.r. and locks the channels in an open state [19, 20]. Ryanodine does not alter the Ca^{2+} sensitivity of the contractile elements, which is proven in skinned preparations [21]. If the Ca^{2+} release channels are locked in the open state, Ca^{2+} leaks out from the channels even though Ca^{2+} is taken up by the Ca^{2+} pump of the s.r. Thus, Ca^{2+} cannot be accumulated in the s.r. Therefore, the Ca^{2+} content of the s.r. decreases and the Ca^{2+} gradient across the s.r. membrane is subsequently diminished. As a result, the peak of the Ca^{2+} signal in twitch contraction is decreased in accordance with the prolonged time course. Tension is significantly diminished and also accompanies the prolonged time course. The prolonged time course of tension is mainly explained by the prolongation of Ca^{2+} transient. This significantly small tension compared with that in the absence of the drug might cause the prolongation of the time course of Ca^{2+} transients and shorten the relaxation time, as a result of a tension-dependent feedback mechanism (see Downstream section). However, the

Fig. 4. Effects of $[Ca^{2+}]_o$ (2, 4, and 8 mM) on Ca^{2+} transients and tension in ferret papillary muscles. In D, the Ca^{2+} transients and tension are normalized to match their peaks and superimposed to show the changes in the time courses of both signals. Aequorin light signal is expressed as fractional luminescence (L_f). Sixty-four signals were averaged.

slower decay of Ca^{2+} transients overcomes the faster relaxation which is thought to be due to the tension-dependent feedback mechanism.

Caffeine rapidly diffuses into the myoplasm [22] and directly releases Ca^{2+} from the s.r. by enhancing the Ca^{2+} -induced Ca^{2+} release mechanism [23]; caffeine also increases the apparent Ca^{2+} sensitivity of the contractile elements, which has been proven in skinned preparations [24]. In addition to the Ca^{2+} releasing mechanism, caffeine prolongs the time course of Ca^{2+} transient and this is considered to be due to the slower Ca^{2+} uptake by the s.r., although the mechanism is not fully understood [25]. An increase in the apparent Ca^{2+} sensitivity should prolong the relaxation time and shorten the decay time of Ca^{2+} transients. However, the slower uptake of Ca^{2+} by the s.r. probably masks the shortening of the decay of Ca^{2+} transients.

2) *Inhibitors of Ca^{2+} pump in s.r.* Thapsigargin is known as a specific inhibitor of the Ca^{2+} pump in the s.r. and is widely used [26]. When thapsigargin is applied to cardiac myocytes, the time courses of cell length shortening and Ca^{2+} signal are prolonged and their peaks are decreased [26–28]. When the rate of Ca^{2+}

uptake is decreased by thapsigargin, Ca^{2+} is not sufficiently taken up by the s.r. within the limited time and the available Ca^{2+} for the following contraction is decreased. Thus, the Ca^{2+} transient is decreased with a slower decay time. In contrast to single isolated myocytes, thapsigargin does not alter the magnitude or the time course of tension in papillary muscles [29]. We measured the Ca^{2+} transients of aequorin from the superficial cells of a papillary muscle with tension in the presence and absence of thapsigargin. The Ca^{2+} transient was clearly decreased with a prolonged time course but the tension was scarcely altered by thapsigargin. The Ca^{2+} sensitivity of the myofilaments in skinned preparations was not significantly altered by thapsigargin [30]. The reason why the tension of the multicellular preparation is not influenced by thapsigargin as in the Ca^{2+} transients is not clear. One possible explanation is that thapsigargin hardly diffuses into the core of the preparation due to unknown mechanisms.

3) *Ca^{2+} overload.* When $[\text{Ca}^{2+}]_i$ increases over the Ca^{2+} uptake capacity of the s.r., oscillation of the Ca^{2+} signal occurs, with a frequency of about 3 Hz at 30°C [31, 32]. This oscillation is caused by the spontaneous Ca^{2+} release from the s.r., because the oscillation can be blocked by ryanodine [32]. Ca^{2+} oscillations at the resting state and after contraction are observed under the following conditions: high rate of electrical stimulation, solutions with high $[\text{Ca}^{2+}]_o$, Na^+ -deficient solutions, β -adrenoceptor stimulation with isoprenaline, phosphodiesterase inhibitors, and the inhibition of Na-K pump by cardiac glycosides. These Ca^{2+} oscillations occur particularly with a combination of these factors (i.e., isoprenaline plus high rate of stimulation or high $[\text{Ca}^{2+}]_o$ plus ouabain etc.) [14, 33]. In the presence of Ca^{2+} oscillation, cells of multicellular preparations contract asynchronously [32], which produces less tension compared to that when all cells in the preparation contract synchronously in the absence of Ca^{2+} oscillation. Therefore, the Ca^{2+} -overload condition seriously influences the developed tension. Asynchronous contraction and relaxation of some cells in the preparation produces an inhomogeneity of $[\text{Ca}^{2+}]_i$ in the preparation. Thus, the state of the $[\text{Ca}^{2+}]_i$ before stimulation is an important factor which determines the subsequent contractions in multicellular preparations.

CENTRAL

Many factors are known to influence the Ca^{2+} binding to troponin-C either directly or indirectly. If the Ca^{2+} binding to troponin-C is altered, tension development at the same $[\text{Ca}^{2+}]$ is changed (the change in the Ca^{2+} sensitivity of the contractile elements). However, many steps are involved in tension development after an increase in $[\text{Ca}^{2+}]_i$. Therefore, the change in the Ca^{2+} sensitivity of the contractile elements is not restricted to the change in the affinity of troponin-C for Ca^{2+} . Here, " Ca^{2+} sensitivity" is used to express the responsiveness of the contractile elements to Ca^{2+} including the change in the affinity of troponin-C for Ca^{2+} .

The evaluation of the Ca^{2+} sensitivity of the contractile elements in the intact cell is not fully established [6]. Several methods are employed for the evaluation of the Ca^{2+} sensitivity of the contractile elements in intact preparations: i) the relation between the peak of the Ca^{2+} signal and the tension at the peak of the Ca^{2+} signal in twitch, ii) the relation between the peak of the aequorin light signal and $dP/dt_{\max}$ in twitch, iii) instantaneous relation between $[\text{Ca}^{2+}]_i$ and dP/dt in twitch, and iv) the relation between $[\text{Ca}^{2+}]_i$ and tension in Na^+ -deficient contracture and tetanization [14, 17, 33–36]. Mammalian cardiac muscles can be tetanized, if the preparation is treated with ryanodine and repetitive stimulation is applied [35, 36]. In tetanic contraction, changes in $[\text{Ca}^{2+}]_o$ affect $[\text{Ca}^{2+}]_i$ and tension in a graded manner. Thus, the relation between $[\text{Ca}^{2+}]_i$ and tension at a quasi-steady state can be measured [35, 36]. The measurement of the relation between $[\text{Ca}^{2+}]_i$ and tension in tetanic contraction is one of the possible methods to evaluate the changes in the Ca^{2+} sensitivity of the contractile elements in intact preparations. An advantage of the tetanizing method is that the change in the Ca^{2+} sensitivity which is induced by second messengers (cAMP, for example, is known to decrease the Ca^{2+} sensitivity and cGMP is suggested to have an opposite effect—see the section of β -adrenoceptor stimulation and acetylcholine for details) and by the change in the intracellular ionic environment can be evaluated in intact preparations. This is not feasible in skinned preparations unless specific substrates and ions which are expected to be altered are added to the bathing solutions.

Ca^{2+} binding to troponin-C is a crucial step in the Central mechanism. The time course of the troponin- Ca^{2+} complex can be calculated using the fixed on- and off-rate constants of troponin-C for Ca^{2+} following the model of Robertson *et al.* [13] (Fig. 3). However, if the affinity of troponin-C for Ca^{2+} is altered through the Central mechanism or through the feedback mechanism (discussed later in the Downstream section), $[\text{Ca}^{2+}]_i$ is greatly influenced. If the affinity of troponin-C is changed, the off-rate of Ca^{2+} from troponin-C is considered to be primarily altered, because the on-rate is considered to be limited by the diffusion rate of Ca^{2+} in the myoplasm.

Some factors known to influence the apparent Ca^{2+} sensitivity of the contractile elements are discussed below.

1. Effects of intracellular ions

1) pH_i . If the cardiac muscle is exposed to a higher concentration of CO_2 , tension is decreased even though there is a sufficient supply of Ca^{2+} [37–40]. Therefore, the contractile apparatus cannot respond to Ca^{2+} . The peak of the Ca^{2+} transient in twitch is increased and the time course is prolonged. Tension is dramatically suppressed but the time course of tension is not significantly altered [38, 40]. However, one report stated a slight shortening of the relaxation time [41]. The main mechanism of CO_2 acidosis is a decrease in pH_i (intracellular acidification) and similar effects are observed in metabolic acidosis in which the concentration of bicarbonate is lowered [37, 40]. Intracellular acidification influ-

ences many cellular functions such as, Ca^{2+} channels, Ca^{2+} release channels of the s.r., and Ca^{2+} pump of the s.r., Ca^{2+} binding to troponin-C [42–45]. Therefore, the changes in the Ca^{2+} transients and contractile properties are caused by these factors. The inhibition of the Ca^{2+} current and Ca^{2+} release channels by H^+ should decrease the peak of the Ca^{2+} transients [42–44]. However, the peak of the Ca^{2+} transients is increased by intracellular acidification [37–40]. Therefore, factors other than the Ca^{2+} current and Ca^{2+} release channels must be involved in the increase in the Ca^{2+} transients. An increase in $[\text{H}^+]_i$ decreases the Ca^{2+} binding to troponin-C, which occurs as a secondary effect of H^+ on troponin-I or troponin-T [45, 46]. When Ca^{2+} binding to troponin-C and the rate of Ca^{2+} uptake by the s.r. are decreased, $[\text{Ca}^{2+}]_i$ is not sufficiently lowered compared to the control, which alternatively increases the peak of Ca^{2+} transient and prolongs the time course.

In CO_2 acidosis, $[\text{Ca}^{2+}]_i$ -tension relation of tetanic contraction is shifted to the right and the maximal tension is decreased (Fig. 5). The rightward shift of the $[\text{Ca}^{2+}]_i$ -tension relation is clearly observed if the maximal tension levels in the control and in acidosis are normalized to match. The slope of the relation (Hill coefficient) indicates the cooperativity of the contractile elements. In CO_2 acidosis, no significant change of the Hill coefficient is observed in intact preparations (unpublished observation), although the evaluation of a slight change in the Hill

Fig. 5. Relation between $[\text{Ca}^{2+}]_i$ and tension measured using tetanic contraction at different CO_2 concentrations; at 5% CO_2 pH_o was 7.34, and at 15% CO_2 pH_o was 6.89. In CO_2 acidosis, maximal tension is decreased and the relation is shifted to the right. The normal Tyrode's solution contains 20 mM NaHCO_3 (unpublished data).

coefficient is difficult. A decrease in the Ca^{2+} sensitivity of the contractile elements and suppression of the maximal tension caused by a decrease in pH_i have also been shown using skinned preparations [46, 47].

If intracellular acidification decreases the Ca^{2+} sensitivity of the contractile elements, Ca^{2+} is dissociated from troponin-C at a faster rate, which shortens the relaxation time. A marked decrease in the developed tension in intracellular acidification probably further decreases the affinity of troponin-C for Ca^{2+} , which accelerates relaxation. However, the shortening of the relaxation time is slight and cannot always be observed [40]. We consider that the prolongation of the decay time of the Ca^{2+} transients due to the slower Ca^{2+} uptake by s.r. [48, 49] prolongs relaxation, which is the opposite of the faster relaxation expected from the decrease in the Ca^{2+} sensitivity. As a whole effect, the relaxation time might not be significantly shortened in intracellular acidification. Thus, intracellular acidification influences various cell functions but the marked reduction of the developed tension is due to decreased Ca^{2+} sensitivity, which might alternatively influence the Ca^{2+} transient.

2) $[\text{Mg}^{2+}]_i$. Mg^{2+} is one of the factors which decreases the Ca^{2+} sensitivity of the myofilaments, as confirmed in skinned preparations [50, 51]. Intracellular Mg^{2+} is suggested to modulate various cellular functions (ionic channels, Ca^{2+} release mechanism, Ca^{2+} sensitivity of the contractile elements, etc.) [50–54]. $[\text{Mg}^{2+}]_i$ can now be measured using the Mg^{2+} indicator, fura-2, under various conditions [55, 56]. However, $[\text{Mg}^{2+}]_i$ is hardly altered by most interventions, with the exception of metabolic inhibition [56]. Therefore, the modulation of contractile properties by intracellular Mg^{2+} might be important when metabolism is inhibited and intracellular ATP is seriously depleted in ischemia or hypoxia [57].

3) *Inorganic phosphate*. Inorganic phosphate is known to decrease the Ca^{2+} sensitivity of the contractile elements as well as the maximal tension [46, 58]. The concentration of inorganic phosphate rises when ATP hydrolysis is enhanced during ischemia and hypoxia. Therefore, inorganic phosphate plays an important role in characterizing the contraction under pathophysiological conditions [46, 58].

2. *Effects of β -adrenoceptor stimulation*

Stimulation of β -adrenoceptors is known to modulate the intracellular Ca^{2+} transients and contractile properties [14, 18, 33, 36], which are caused by phosphorylation of the target proteins of cAMP-dependent protein kinase (A-kinase): Ca^{2+} channels, phospholamban of the s.r., and troponin-I [59–61]. Therefore, the effect of β -adrenoceptor stimulation cannot be limited to the Central mechanism, the Ca^{2+} binding to troponin-C. The peaks of Ca^{2+} transients and tension increase concomitantly with faster time courses [14, 18, 33, 36]. The faster time to peak tension is partly due to the faster time to peak Ca^{2+} signal but the faster cross-bridge cycling rate is an additional factor involved in the determination of the time course [62]. The shorter relaxation time induced by β -adrenoceptor stimulation has been explained by faster Ca^{2+} uptake by the s.r. (due to the phosphorylation of

phospholamban) and a decrease in the Ca^{2+} sensitivity of the contractile elements (due to the phosphorylation of troponin-I). However, the changes in the relaxation time do not parallel the changes in these two parameters or the cross-bridge cycling rate [63]. There is a possibility that other factors are involved in the regulation of relaxation.

The relation between $[\text{Ca}^{2+}]_i$ and tension in tetanic contraction, which is one means of evaluating Ca^{2+} sensitivity, is clearly shifted to the right and the Hill coefficient (cooperativity) is decreased by isoprenaline (Fig. 6) [36, 63]. Therefore, β -adrenoceptor stimulation decreases the apparent Ca^{2+} sensitivity of the contractile elements and the cooperativity. The molecular mechanisms of these changes are not fully understood but the phosphorylation of troponin-I is suggested to influence the Ca^{2+} binding to troponin-C [61, 64]. Thus, the tetanization method can demonstrate the changes in the Ca^{2+} sensitivity and the cooperativity induced by the stimulation of β -adrenoceptor, which also influences the Upstream mechanism (Ca^{2+} transient). In β -adrenoceptor stimulation, peak tension is increased, because the increase in the Ca^{2+} transient overcomes the decrease in the Ca^{2+} sensitivity. The potentiation of tension is dependent upon the range of $[\text{Ca}^{2+}]_i$ which activates twitch contraction. If the developed tension before β -adrenoceptor stimulation is near the maximal level which the preparation can

Fig. 6. Effects of isoprenaline on the relation between $[\text{Ca}^{2+}]_i$ and tension in tetanic contraction (see Okazaki *et al.* [36] for details).

produce, the potentiating effects on contraction is small. This hypothesis could explain the different twitch potentiating effects of β -adrenoceptor stimulation in different specimens [18, 33, 36].

3. *Acetylcholine*

Acetylcholine is known to antagonize the effects of β -adrenoceptor stimulation on cardiac muscle contraction. The altered Ca^{2+} transient induced by β -adrenoceptor stimulation is restored to control before stimulation by acetylcholine [63, 65]. The altered time course of tension by β -adrenoceptor stimulation, except for the relaxation time, is also recovered to the control by acetylcholine. The decreased Ca^{2+} sensitivity of the contractile elements induced by β -adrenoceptor stimulation is recovered by the addition of acetylcholine, which has been proven using the tetanization method [63]. However, the shortened relaxation time by β -adrenoceptor stimulation does not recover to control before stimulation although the other parameters of the Ca^{2+} transient and tension as well as the cross-bridge cycling rate are all restored by acetylcholine [63].

cGMP is considered to be a factor mediating the effects of muscarinic receptor stimulation in the rat ventricular muscles which are treated with a high concentration of EDTA [66]. However, we failed to observe the effects of nitroprusside and the membrane permeable type cGMP on the Ca^{2+} transients and tension in intact ferret ventricular muscles [67]. Therefore, the inhibition of adenylate cyclase through G (probably G_i) protein is the primary mechanism of muscarinic receptor stimulation in ferret ventricular muscles [67].

4. *Adenosine*

Adenosine is released in ischemia and has protective effects on cardiac muscles [68]. Adenosine slightly decreases the peak of the Ca^{2+} transient and prolongs the time course. The peak developed tension is maintained even though the peak of the Ca^{2+} transient is decreased. These effects of adenosine are more clearly observed when the preparation is treated with isoprenaline before the application of adenosine. The relation between $[\text{Ca}^{2+}]_i$ and tension in tetanic contraction is shifted to the left by adenosine in the presence or absence of isoprenaline [69]. Therefore, a possible explanation of the effects of adenosine is the inhibition of adenylate cyclase activity, which lowers the cAMP concentration and dephosphorylates the target proteins of A-kinase. However, a different mechanism has also been suggested in ferret ventricular muscles [70].

DOWNSTREAM

The process following the Ca^{2+} binding to troponin-C, in particular the cross-bridge attachment, is another important step in the cardiac muscle contraction.

1. *Muscle length*

The developed tension of cardiac muscle highly depends on muscle length (see Allen and Kentish [71] for the review). Allen and Kurihara [72] measured Ca^{2+} transients at different muscle lengths; the changes in the peak tension at different muscle lengths do not accompany the change in the peak of the Ca^{2+} transient but the decay time of Ca^{2+} transient is altered. These changes are observed immediately after changing muscle length. The peak of the Ca^{2+} transient is altered at a later stage of the length change. They postulated that the developed tension at different muscle lengths influences the affinity of troponin-C for Ca^{2+} , which subsequently alters the Ca^{2+} transients. This hypothesis (the tension-dependent change in the affinity of troponin-C for Ca^{2+}) was further supported by measurement of the $[\text{Ca}^{2+}]$ change in the bathing solution of the Triton X-treated skinned preparations with alteration of the muscle length in the preparation activated by a low concentration of Ca^{2+} [73].

2. *Quick release and stretch*

In intact preparations, a quick release of muscle which is applied during a twitch contraction transiently increases $[\text{Ca}^{2+}]_i$ in the falling phase of Ca^{2+} transients (extra- Ca^{2+}) [72]. Similar changes of the Ca^{2+} transient are observed in active shortening [74]. Therefore, the time course of the Ca^{2+} transient in cell length shortening in a single myocyte differs from that in tension measurement. However, muscle length change does not alter Ca^{2+} transients when tension is inhibited by 2,3-butanedione monoxime (BDM) (Fig. 7) [75]. Therefore, the alteration of tension is essential for producing the extra- Ca^{2+} . The sudden reduction of active tension during contraction, due to the cross-bridge detachment, decreases the affinity of troponin-C, which transiently increases $[\text{Ca}^{2+}]_i$. Thus, the tension-dependent change in the affinity of troponin-C for Ca^{2+} is observed in intact preparations. This tension-dependent change of the affinity of troponin-C was also recognized when we measured the changes in Ca^{2+} signals in tetanic contraction and in preparations treated with a high concentration of ouabain and Ca^{2+} [76]. The tension-dependent change of the Ca^{2+} binding to myofilaments was also found using skinned preparations [77, 78].

Extra- Ca^{2+} can be observed even though the s.r. function is inhibited by caffeine, ryanodine, and thapsigargin (unpublished data). In addition, extra- Ca^{2+} appears when muscle length is quickly shortened during rapid cooling contracture which is caused by the Ca^{2+} release from the s.r. without an accompanying influx of Ca^{2+} (Tanaka and Kurihara, unpublished observation). Therefore, the origin of the extra- Ca^{2+} is not Ca^{2+} influx through the cell membrane and is not the change in the Ca^{2+} handling in the s.r. The troponin-Ca complex which follows the Ca^{2+} transient is a possible origin of the extra- Ca^{2+} .

A similar extra- Ca^{2+} is also reported in barnacle muscles [79]. In barnacle muscles, the time course of the magnitude of the extra- Ca^{2+} (profile of the extra- Ca^{2+}) immediately follows the time course of the Ca^{2+} signal, suggesting that

Fig. 7. Effects of 2,3-butanedione monoxime (BDM) on extra- Ca^{2+} in response to quick releases [75]. The muscle was quickly shortened during contraction in the presence (E, F) or absence of BDM (B, C). In G, records of light signals in the control are superimposed. In H, records of light signals in the presence of BDM are superimposed. Light signals are expressed as current of a photomultiplier. Sixty-four signals were averaged. Arrows indicate extra- Ca^{2+} .

the origin of the extra- Ca^{2+} is the troponin-Ca complex. Force development of the barnacle muscle immediately follows the Ca^{2+} signal and the time courses of both signals are closer to each other compared to those in cardiac muscles. The time lag between the Ca^{2+} signal and tension, which differs depending on muscle specimen, probably characterizes the extra- Ca^{2+} produced in response to mechanical perturbations. If the time lag between the Ca^{2+} transient which just precedes the

troponin- Ca complex (the origin of extra- Ca^{2+}) and tension is short, a change in tension can be applied to a higher level of Ca^{2+} transient in which more troponin- Ca complex is formed and more extra- Ca^{2+} is produced.

Allen and Kurihara [72] could not detect a significant change in the Ca^{2+} transient when the muscle was stretched during a twitch contraction. A similar asymmetrical change in the Ca^{2+} signal in release and stretch is recognized in tetanized preparations [76]. When the length of tetanized muscle is quickly shortened, a spike-like increase in the Ca^{2+} signal is observed. However, the spike-like change in the Ca^{2+} signal cannot be observed when the muscle length is restored to the original length before the release. The restoration of muscle length produces both a decrease in the Ca^{2+} signal and tension development with a slower time course (delayed activation). The time courses of both signals (Ca^{2+} signal and tension) are similar and the direction of the change is opposite. These results suggest that the change in the affinity of troponin-C for Ca^{2+} appears in an asymmetrical manner in release and stretch. The asymmetry of the change in Ca^{2+} signals in release and stretch might be explained by the following hypothesis; if the cross-bridges are forcibly detached by stretching the muscle, active tension is not sufficiently produced and the tension-dependent change of the affinity of troponin-C for Ca^{2+} does not occur. Thus, Ca^{2+} transient is not influenced if active tension is not produced by the forcible detachment of the cross-bridges in stretch. However, in release, the developed active tension can be sufficiently decreased by the cross-bridge detachment. Thus, if the affinity of troponin-C for Ca^{2+} is decreased, which dissociates Ca^{2+} from the binding site on troponin-C, Ca^{2+} transients are altered. Stretching of the barnacle muscle shows biphasic changes of Ca^{2+} transient which are due to the increase in the Ca^{2+} affinity of the activating sites due to cross-bridge attachment and cross-bridge strain [80].

This tension-dependent change in the affinity of cardiac troponin-C for Ca^{2+} which is observed in intact and skinned preparations is probably an important mechanism in the determination of the magnitude and the time course of contraction under physiological conditions. As indicated in the Upstream section, the time courses of tension and Ca^{2+} transients are altered when $[\text{Ca}^{2+}]_o$ is simply changed. The changes of the time course of the Ca^{2+} transient at different $[\text{Ca}^{2+}]_o$ might partly be explained by the changes in the affinity of troponin-C for Ca^{2+} in a tension-dependent manner, although the effects of $[\text{Ca}^{2+}]_o$ on the decay of the Ca^{2+} transient through membrane currents and Ca^{2+} release mechanisms should also be considered to be involved. If the decay of the Ca^{2+} transient is primarily altered by the effect of $[\text{Ca}^{2+}]_o$, the relaxation time is expected to alter in the same direction as the change in the falling phase of the Ca^{2+} transient. However, the change in the relaxation time is opposite to that of the change in the falling phase of the Ca^{2+} transient. If more active tension is developed at higher $[\text{Ca}^{2+}]_o$, the affinity of troponin-C for Ca^{2+} increases and more Ca^{2+} binds to troponin-C, which shortens the falling phase of the Ca^{2+} transient and prolongs relaxation. The opposite is also true. If the developed tension is decreased when the magnitude of Ca^{2+} transient

is decreased by an Upstream mechanism, the decreased tension further reduces the affinity of troponin-C for Ca^{2+} , which further diminishes tension. This mechanism secondarily alters the Ca^{2+} transients. Therefore, the tension-dependent change in the affinity of troponin-C works under physiological conditions. This feedback mechanism could explain the steepness of the ascending limb of the length-tension relation in cardiac muscles, although changes in length have also been suggested to be involved [81].

SUMMARY

In this short review, some of the factors which influence the intracellular Ca^{2+} transients and contraction in mammalian cardiac muscles which we have been examining are discussed. The processes concerning the change in $[\text{Ca}^{2+}]_i$ and contraction are divided into three steps following the scheme proposed by Blinks [6]: Upstream, Central, and Downstream. However, these processes are interrelated. Although the Ca^{2+} transient is an important step for triggering contraction, it is influenced by the following Central and Downstream mechanisms. The evaluation of Ca^{2+} sensitivity of the contractile elements (Central mechanism) in intact preparations is discussed; measurement of the relation between $[\text{Ca}^{2+}]_i$ and tension in tetanic contraction is one of the possible methods. A change in the Ca^{2+} binding to troponin-C is an important factor to determine the Ca^{2+} sensitivity but other mechanisms are also considered to be involved. In cardiac muscles, the feedback mechanism from the Downstream, in particular the cross-bridge attachment and detachment, to the Central processes is a physiologically important mechanism in the determination of contractile properties. This feedback mechanism alternatively influences the intracellular Ca^{2+} transient by changing the affinity of troponin-C for Ca^{2+} . Thus, the interpretation of the measured Ca^{2+} transients is not simple and the numerous factors involved must be considered. Further study is necessary to elucidate the molecular mechanisms which are responsible for the feedback from the Downstream mechanism to the Central and Upstream mechanisms.

Key words: Ca^{2+} , Ca^{2+} transient, cardiac muscle, muscle length.

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REFERENCES

1. Ebashi S and Endo M: Calcium ion and muscle contraction. *Prog Biophys Mol Biol* **18**: 123-183, 1968

2. Fabiato A: Calcium release in skinned cardiac cells: Variations with species, tissues, and development. *Fed Proc* **41**: 2238–2244, 1982
3. Blinks JR, Wier WG, Hess P, and Prendergast FG: Measurement of Ca^{2+} concentrations in living cells. *Prog Biophys Mol Biol* **40**: 1–114, 1982
4. Holroyde MJ, Robertson SP, Johnson JD, Solaro RJ, and Potter JD: 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, 1980
5. Cheung WY: Calmodulin plays a pivotal role in cellular regulation. *Science* **207**: 19–27, 1980
6. Blinks JR: Analysis of the effects of drugs on myofibrillar Ca^{2+} sensitivity in intact cardiac muscle. *In: Modulation of Cardiac Calcium Sensitivity*, ed. Lee JA and Allen DG, Oxford University Press, New York, pp 242–282, 1993
7. Grynkiewicz G, Poenie M, and Tsien R: A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* **260**: 3440–3450, 1985
8. Spurgeon HA, Stern MD, Baartz G, Raffaeli S, Hansford RG, Talo A, Lakatta EG, and Capogrossi MC: Simultaneous measurement of Ca^{2+} , contraction and action potential in cardiac myocytes. *Am J Physiol* **258**: H574–H586, 1990
9. Backx PH and Ter Keurs HEDJ: Fluorescent properties of rat trabeculae microinjected with fura-2 salt. *Am J Physiol* **264**: H1098–H1110, 1993
10. Baylor SM and Hollingworth S: Fura-2 calcium transients in frog skeletal muscle fibres. *J Physiol (Lond)* **403**: 151–192, 1988
11. Suda N and Kurihara S: Intracellular calcium signals measured with fura-2 and aequorin in frog skeletal muscle fibers. *Jpn J Physiol* **41**: 277–295, 1991
12. Konishi M, Olson A, Hollingworth S, and Baylor SM: Myoplasmic binding of fura-2 investigated by steady-state fluorescence and absorbance measurements. *Biophys J* **54**: 1089–1104, 1988
13. Robertson SP, Johnson JD, and Potter JD: 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, 1981
14. Allen DG and Kurihara S: Calcium transients in mammalian ventricular muscle. *Eur Heart J (Suppl A)* **1**: 5–15, 1980
15. Beuckelmann DJ and Wier WG: Mechanism of release of calcium from sarcoplasmic reticulum of guinea-pig cardiac cells. *J Physiol (Lond)* **405**: 233–255, 1988
16. Berlin JR and Konishi M: Ca^{2+} transients in cardiac myocytes measured with high and low affinity Ca^{2+} indicators. *Biophys J* **65**: 1632–1647, 1993
17. Sipido K and Wier WG: Flux of Ca^{2+} across the sarcoplasmic reticulum of guinea-pig cardiac cells during excitation-contraction coupling. *J Physiol (Lond)* **435**: 605–630, 1991
18. Endoh M and Blinks JR: Actions of sympathomimetic amines on the Ca^{2+} transients and contractions of rabbit myocardium: Reciprocal changes in myofibrillar responsiveness to Ca^{2+} mediated through α - and β -adrenoceptors. *Circ Res* **62**: 247–265, 1988
19. Meissner G: Ryanodine activation and inhibition of the Ca^{2+} release channel of sarcoplasmic reticulum. *J Biol Chem* **261**: 6300–6306, 1986
20. Inui M, Wang S, Saito A, and Fleischer S: Characterization of junctional and longitudinal sarcoplasmic reticulum from heart muscle. *J Biol Chem* **263**: 10843–10850, 1988

21. Fabiato A: Effects of ryanodine in skinned cardiac cells. *Fed Proc* **44**: 2970–2976, 1985
22. O'Neill SC, Donoso P, and Eisner DA: The role of $[Ca^{2+}]_i$ and $[Ca^{2+}]_i$ sensitization in the caffeine contracture of rat myocytes: Measurement of $[Ca^{2+}]_i$ and $[caffeine]_i$. *J Physiol (Lond)* **425**: 55–70, 1990
23. Endo M: Ca release from the sarcoplasmic reticulum. *Physiol Rev* **57**: 71–108, 1977
24. Wendt IR and Stephenson DG: Effects of caffeine on Ca-activated force production in skinned cardiac and skeletal muscle fibres of the rat. *Pflügers Arch* **398**: 210–216, 1983
25. Weber A and Herz R: The relationship between caffeine contracture of intact muscle and the effect of caffeine on isolated reticulum. *J Gen Physiol* **52**: 750–759, 1968
26. Kirby MS, Sagara Y, Gaa S, Inesi G, Lederer WJ, and Rogers TB: Thapsigargin inhibits contraction and Ca^{2+} transient in cardiac cells by specific inhibition of the sarcoplasmic reticulum Ca^{2+} pump. *J Biol Chem* **267**: 12545–12551, 1992
27. Janczewski AM and Lakatta EG: Thapsigargin inhibits Ca^{2+} uptake, and Ca^{2+} depletes sarcoplasmic reticulum in intact cardiac myocytes. *Am J Physiol* **265**: H517–H522, 1993
28. Bassani JWM, Bassani RA, and Bers DM: Twitch-dependent SR Ca accumulation and release in rabbit ventricular myocytes. *Am J Physiol* **265**: C533–C540, 1993
29. Baudet S, Shaoulian R, and Bers DM: Effects of thapsigargin and cyclopiazonic acid on twitch force and sarcoplasmic reticulum Ca^{2+} content of rabbit ventricular muscle. *Circ Res* **73**: 813–819, 1993
30. Tanaka T, Komukai K, Tanaka E, and Kurihara S: Effects of thapsigargin on aequorin-injected and skinned preparations in ferret ventricular muscles. in submitted
31. Orchard CH, Eisner DA, and Allen DG: Oscillations of intracellular Ca^{2+} in mammalian cardiac muscle. *Nature* **304**: 735–738, 1983
32. Wier WG, Kort AA, Stern MD, Lakatta EG, and Marban E: Cellular calcium fluctuations in mammalian heart. *Proc Natl Acad Sci USA* **80**: 7367–7371, 1983
33. Kurihara S and Konishi M: Effects of β -adrenoceptor stimulation on intracellular Ca transients and tension in rat ventricular muscle. *Pflügers Arch* **409**: 427–437, 1987
34. Yue DT: Intracellular $[Ca^{2+}]$ related to rate of force development in twitch contraction of heart. *Am J Physiol* **252**: H760–H770, 1987
35. Yue DT, Marban E, and Wier WG: Relationship between force and intracellular $[Ca^{2+}]$ in tetanized mammalian heart muscle. *J Gen Physiol* **87**: 223–242, 1986
36. Okazaki O, Suda N, Hongo K, Konishi M, and Kurihara S: Modulation of Ca^{2+} transients and contractile properties by β -adrenoceptor stimulation in ferret ventricular muscles. *J Physiol (Lond)* **423**: 221–240, 1990
37. Allen DG and Orchard CH: The effects of changes of pH on intracellular calcium transients in mammalian cardiac muscle. *J Physiol (Lond)* **335**: 555–567, 1983
38. Kurihara S and Konishi M: Effects of isoproterenol and CO_2 acidosis on intracellular Ca transients and tension in rat ventricular muscle. In: *Cardiac Function under Ischemia and Hypoxia*, ed. Yamada K and Katz AM, The University of Nagoya Press, Nagoya, pp 287–301, 1986
39. Orchard CH, Hamilton DL, Astles P, McCall E, and Jewell BR: The effect of acidosis on the relationship between Ca^{2+} and force in isolated ferret cardiac muscle. *J Physiol (Lond)* **436**: 559–578, 1991
40. Kurihara S, Tanaka E, Hongo K, Suda N, Okazaki O, and Saeki Y: Effects of intracellular acidification on Ca^{2+} transients and contraction in mammalian cardiac

- muscles. *In: The Diabetic Heart*, ed. Nagano M and Dhalla N, Raven Press, New York, pp 515–522, 1991
41. Orchard CH and Kentish JC: Effects of changes of pH on the contractile function of cardiac muscle. *Am J Physiol* **258**: C967–C981, 1990
 42. Kurachi Y: The effects of intracellular protons on the electrical activity of single ventricular cells. *Pflügers Arch* **394**: 264–270, 1982
 43. Fabiato A: Use of aequorin for the appraisal of the hypothesis of the release of calcium from the sarcoplasmic reticulum induced by a change of pH in skinned cardiac cells. *Cell Calcium* **6**: 95–108, 1985
 44. Rousseau E and Pinkos J: pH modulates conducting and gating behaviour of single calcium release channels. *Pflügers Arch* **415**: 645–647, 1990
 45. Solaro RJ, Kumar P, Blanchard EM, and Martin AF: Differential effects of pH on calcium activation of myofilaments of adult and perinatal dog hearts: Evidence for developmental differences in thin filament regulation. *Circ Res* **58**: 721–729, 1986
 46. Kentish JC and Palmer S: The influence of pH, phosphate, and ionic strength on contraction in skinned cardiac muscle. *In: Modulation of Cardiac Calcium Sensitivity: A New Approach to Increasing the Strength of the Heart*, ed. Lee JA and Allen DG, Oxford University Press, New York, pp 67–88, 1993
 47. Fabiato A and Fabiato F: Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *J Physiol (Lond)* **276**: 233–255, 1978
 48. Grassi de Gende AO: The effect of pH on the calcium dependence of calcium accumulation in dog cardiac muscle sarcoplasmic reticulum. *J Mol Cell Cardiol* **20**: 1087–1093, 1988
 49. Kawai M and Konishi M: Measurement of sarcoplasmic reticulum calcium content in skinned mammalian cardiac muscle. *Cell Calcium* **16**: 123–136, 1994
 50. Kerrick WGL and Donaldson SKB: The comparative effects of $[\text{Ca}^{2+}]$ and $[\text{Mg}^{2+}]$ on tension generation in the fibers of skinned frog skeletal muscle and mechanically disrupted rat ventricular cardiac muscle. *Pflügers Arch* **385**: 195–201, 1975
 51. Fabiato A and Fabiato F: Effects of magnesium on contractile activation of skinned cardiac cells. *J Physiol (Lond)* **249**: 497–517, 1975
 52. White RE and Hartzell HC: Magnesium ions in cardiac function. Regulator of ion channels and second messengers. *Biochem Pharmacol* **38**: 859–867, 1989
 53. Matsuda H, Saigusa A, and Irisawa H: Ohmic conductance through the inwardly rectifying K channel and blocking by internal Mg^{2+} . *Nature* **325**: 156–159, 1987
 54. Romani A and Scarpa A: Hormonal control of Mg^{2+} transport in the heart. *Nature* **346**: 841–844, 1990
 55. Silverman HS, Di Lisa F, Hui RC, Miyata H, Sollot SJ, Hansford RG, Lakatta EG, and Stern MD: Regulation of intracellular free Mg^{2+} and contraction in single adult mammalian cardiac myocytes. *Am J Physiol* **266**: C222–C233, 1994
 56. Hongo K, Konishi M, and Kurihara S: Cytoplasmic free Mg^{2+} in rat ventricular myocytes studied with the fluorescent indicator fura-2. *Jpn J Physiol* **44**: 357–378, 1994
 57. Kirkels JH, van Echteld CJA, and Ruigrok TJC: Intracellular magnesium during myocardial ischemia and reperfusion: Possible consequences for post ischemic recovery. *J Mol Cell Cardiol* **21**: 1209–1218, 1989

58. Kentish JC: The effects of inorganic phosphate and creatine phosphate on force production in skinned muscles from rat ventricle. *J Physiol (Lond)* **370**: 585-604, 1986
59. Reuter H, Cachelin JE, DePeyer JE, and Kokubun S: Modulation of calcium channels in cultured cells by isoproterenol and 8-bromo-cAMP. *Cold Spring Harbor Symp Quant Biol* **48**: 193-200, 1983
60. Tada M and Katz AM: Phosphorylation of the sarcoplasmic reticulum and sarcolemma. *Annu Rev Physiol* **44**: 401-423, 1982
61. Ray KP and England PJ: Phosphorylation of the inhibitory subunit of troponin and its effect on the calcium dependence of cardiac myofibril adenosine triphosphatase. *FEBS Lett* **70**: 11-16, 1976
62. Ho JFY, Rossmann GH, Kwan LJ, and Hamilton AM: Adrenaline increases the rate of cycling of crossbridges in rat cardiac muscle as measured by pseudo-random binary noise-modulated perturbation analysis. *Circ Res* **62**: 452-461, 1988
63. Hongo K, Tanaka E, and Kurihara S: Alterations in contractile properties and Ca^{2+} transients by β - and muscarinic receptor stimulation in ferret myocardium. *J Physiol (Lond)* **461**: 167-184, 1993
64. Robertson SP, Johnson JD, Holroyde MJ, Kranias EG, Potter JD, and Solaro RJ: The effect of troponin I phosphorylation on the Ca^{2+} -binding properties of the Ca^{2+} -regulatory site of bovine cardiac troponin. *J Biol Chem* **257**: 260-263, 1982
65. McIvor ME, Orchard CH, and Lakatta EG: Dissociation of changes in apparent myofibrillar Ca^{2+} sensitivity and twitch relaxation induced by adrenergic and cholinergic stimulation in isolated ferret cardiac muscle. *J Gen Physiol* **92**: 509-529, 1988
66. Winegrad S: Regulation of cardiac contractile proteins: Correlation between physiology and biochemistry. *Circ Res* **55**: 565-574, 1984
67. Hongo K, Tanaka E, and Kurihara S: Mechanism of the effects of acetylcholine on the contractile properties and Ca^{2+} transients in ferret ventricular muscles. *J Physiol (Lond)* **461**: 185-199, 1993
68. Kitakaze M, Hori M, Takashima S, Sato H, Inoue M, and Kamada T: Ischemic preconditioning increases adenosine release and 5'-nucleotidase activity during myocardial ischemia and reperfusion in dogs. *Circ Res* **87**: 208-215, 1993
69. Komukai K and Kurihara S: Effects of adenosine on Ca^{2+} transients and tension in aequorin-injected ferret papillary muscles. *Pflügers Arch* **428**: 357-363, 1994
70. Endoh M, Takanashi M, Norota Y, Kawabata Y, and Asano T: Pronounced direct inhibitory action mediated by adenosine A_1 receptor and pertussis toxin-sensitive G protein on the ferret ventricular contraction. *Naunyn-Schmiedeberg's Arch Pharmacol* **348**: 282-289, 1993
71. Allen DG and Kentish JC: The cellular basis of the length-tension relation in cardiac muscle. *J Mol Cell Cardiol* **17**: 821-840, 1985
72. Allen DG and Kurihara S: The effects of muscle length on intracellular calcium transients in mammalian cardiac muscle. *J Physiol (Lond)* **327**: 79-94, 1982
73. Allen DG and Kentish JC: Calcium concentration in the myoplasm of skinned ferret ventricular muscle following changes in muscle length. *J Physiol (Lond)* **407**: 489-503, 1988
74. Housmans PR, Lee NKM, and Blinks JR: Active shortening retards the decline of the intracellular calcium transient in mammalian heart muscle. *Science* **221**: 159-161, 1983
75. Kurihara S, Saeki Y, Hongo K, Tanaka E, and Suda N: Effects of length change on

- intracellular Ca^{2+} transients in ferret ventricular muscle treated with 2,3-butanedione monoxime (BDM). *Jpn J Physiol* **40**: 915–920, 1990
76. Saeki Y, Kurihara S, Hongo K, and Tanaka E: Alterations in intracellular calcium and tension of activated ferret papillary muscle in response to step length changes. *J Physiol (Lond)* **463**: 291–306, 1993
 77. Pan B-S and Solaro RJ: Calcium-binding properties of troponin C in detergent-skinned heart muscle fibers. *J Biol Chem* **262**: 7839–7849, 1987
 78. Hofmann PA and Fuchs F: Evidence for a force-dependent component of calcium binding to cardiac troponin C. *Am J Physiol* **253**: C541–C546, 1987
 79. Ridgway EB and Gordon AM: Muscle calcium transient: Effect of post-stimulus length changes in single fibers. *J Gen Physiol* **83**: 75–103, 1984
 80. Gordon AM and Ridgway EB: Stretch of active muscle during the declining phase of the calcium transient produces biphasic changes in calcium binding to the activating sites. *J Gen Physiol* **96**: 1013–1035, 1990
 81. Babu A, Sonnenblick E, and Gulati J: Molecular basis for the influence of muscle length on myocardial performance. *Science* **240**: 74–76, 1988