Alterations in contractile properties and Ca$^{2+}$ handling in streptozotocin-induced diabetic rat myocardium

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Ishikawa, Tetsuya, Hidetoshi Kajiwara, and Satoshi Kurihara. Alterations in contractile properties and Ca$^{2+}$ handling in streptozotocin-induced diabetic rat myocardium. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H2185–H2194, 1999.—The mechanisms of the slower time courses of Ca$^{2+}$ transients (CaT) and contraction in diabetic (diabetes mellitus, DM) myocardium were studied. The aequorin method was applied to papillary muscles of streptozotocin-induced DM and control rats. The time courses of CaT and tension of twitch in DM were slower than those in control, although the magnitudes of the CaT and contraction were identical. The dependence of CaT decay time and relaxation time on developed tension in DM and control rats differed. The length-tension relation in twitch and the pCa-tension relation in tetanus were identical in the two groups. The magnitude of extra Ca$^{2+}$ (transient increase in intracellular Ca$^{2+}$ concentration induced by a quick release in tetanus) was identical in both groups. pCa-tension relations of skinned trabeculae at different sarcomere lengths were nearly identical. The cross-bridge cycling rate (CCR) in DM was slower than that in control. These results indicate that the tension-dependent change in the Ca$^{2+}$ affinity of troponin C in DM myocardium functions as in control myocardium. The slower time courses of CaT and tension in DM myocardium are caused by slower Ca$^{2+}$ uptake by the sarcoplasmic reticulum and the slower CCR.

aequorin; cross-bridge cycling rate; sarcoplasmic reticulum; troponin C; diabetes mellitus

Diabetes mellitus (DM) impairs various functions in the cardiovascular system. Diabetic cardiomyopathy is one of the complications in DM. To clarify the functional changes of diabetic cardiomyopathy, the streptozotocin (STZ)-induced diabetic rat is now widely used as an animal model of DM. The most significant functional change in isolated papillary muscles excised from STZ-induced diabetic rat heart is slower contraction (especially prolonged relaxation) without a change in peak developed tension (9, 23, 33). The slower time course of contraction is attributed to the slower Ca$^{2+}$ uptake by sarcoplasmic reticulum (SR) and to slower cross-bridge cycling (9, 33). However, there are controversial reports regarding the magnitude of contraction and Ca$^{2+}$ transients (CaT) in isolated myocytes in DM. Some reports demonstrated a significantly smaller shortening and CaT in DM compared with those under control conditions (22, 26, 29, 35), but these changes were not proven in another report (34). Thus the molecular mechanisms of the dysfunction in DM myocardium are not fully clarified.

In mammalian cardiac muscles, a change in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) precedes the cross-bridge attachment that leads to tension development. However, the attachment of the cross bridges is considered to influence the affinity of troponin C (TnC) for Ca$^{2+}$, which secondarily alters the decay time of CaT and the relaxation time of contraction (13, 18, 20). The feedback mechanism leads to a steep or more cooperative force-Ca$^{2+}$ relation, so that a given rise in systolic Ca$^{2+}$ produces a larger force than in the absence of this feedback. Therefore, we examined whether the slower relaxation of contraction and the slower decay of CaT in DM are attributable to the dysfunction of SR or the impairment of the tension-dependent feedback mechanism that influences Ca$^{2+}$ binding to TnC.

In the present study, we investigated the mechanisms of slower decay of CaT and slower contraction in STZ-induced DM myocardium, particularly in relation to the tension-dependent change in the affinity of TnC for Ca$^{2+}$. For this purpose, we simultaneously measured CaT and tension under various conditions using the aequorin method. Some of the results were presented at the Japanese Section of the 13th Annual Meeting of the International Society for Heart Research (14).

Materials and Methods

Animals

Eight-week-old male Wistar rats were divided into two groups (control and DM groups). The rats in the DM group were intravenously injected with STZ (50 mg/kg) and were used as the DM group 4–7 wk after the treatment. In the present study, we fixed the period of STZ treatment as almost the same as that in former reports (8, 9, 23, 33) because the state of DM was considered to be significantly altered by the treatment period (6). Age-matched rats without treatment were used as the control group. All animals were kept under the same conditions.

Experiments With Intact Preparations

Rats were anesthetized with pentobarbital sodium (100 mg/kg ip), and after the heart was quickly removed it was connected to the Langendorff apparatus. The blood in the heart was washed out with normal Tyrode solution (see Solutions for Intact Preparations for composition) at 30°C. The heart was then immersed in a bath continuously perfused with normal Tyrode solution at 30 ± 0.5°C. Both ends of the thin papillary muscles or trabeculae were tied with silk threads and dissected from the right ventricle. One end of the preparation was connected to the lever of a motor (CCX-101A, General Scanning, Watertown, MA) that was used to alter muscle length, and the other end was connected to the...
arm of a tension transducer (BG-10, Kulite, Semiconductor Products, Leonia, NJ); compliance 2.5 μm/g; unloaded resonant frequency 1 kHz). The preparation was mounted horizontally in an experimental chamber with a pair of platinum electrodes placed parallel to the preparation for electrical stimulation. The preparation was stimulated with a rectangular pulse at 1.2-fold threshold with a 5-ms duration. The stimulation frequency was 0.2 Hz unless otherwise mentioned. Before the experiment was started, the preparation was slowly stretched from the slack length to the length at which developed tension reached maximum (L_max).

During the stabilization of the preparation, twitch tension in DM preparations was much smaller than that in control and resting tension in DM was higher than that in control. In addition, arrhythmia was frequently induced in DM when the preparation was stretched to obtain L_max. However, the arrhythmia gradually ceased during the course of stabilization, and tension at L_max did not significantly differ between the two groups (Table 1). In control, such arrhythmia and small contractions were not frequently observed. However, if the size of the preparation was small, fibrillation and small contractions occurred even in the control group, which was probably caused by damage to the preparation. The recovery of DM preparation was interesting, but we did not further investigate it in the present study because our main concern was to investigate the alteration of CaT and contraction in DM myocardium by comparing these parameters in control and DM myocardium under the same equilibrated experimental conditions. The muscle length of the DM and control preparations was 2.60 ± 0.59 (mean ± SD; n = 36) and 2.73 ± 0.53 (n = 31) mm, respectively (no significant difference), and the cross-sectional area of the DM and control preparations was 0.248 ± 0.126 (n = 36) and 0.274 ± 0.133 (n = 31) mm², respectively (no significant difference).

Measurement of Intracellular Ca²⁺ With Aequorin

Aequorin injection and measurement of the light signal were essentially similar to that reported previously (13). The light signal of aequorin was converted to [Ca²⁺], using an in vitro calibration curve (2). The constants used in the present experiment were as follows: n, 3.14; Kₚ, 4.025,000; K_T, 114.6 (27). The definitions of the constants have been given previously (2).

Solutions for Intact Preparations

The composition of the normal Tyrode solution used for dissection and for the injection of aequorin was (in mM) 135 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 102 Cl⁻, 20 HCO₃⁻, 1 HPO₄²⁻, 1 SO₄²⁻, 20 acetate, and 10 glucose with 5 U/l insulin (pH 7.35 at 30°C when equilibrated with 5% CO₂-95% O₂). In the experiments, phosphate-free normal Tyrode solution was used to avoid the formation of calcium phosphate when Ca²⁺ concentration was increased in tetanic contraction. Bupranolol (0.1 μM) was also added to the solution to inhibit the effects of endogenous catecholamine. This concentration of bupranolol did not affect the magnitude of developed tension (21). When the Ca²⁺ concentration in the solution was altered, the osmotic pressure of the solution was not adjusted and CaCl₂ was added to or removed from the solution. The temperature of the solution used in the experiments of the intact preparations was continuously monitored with a thermocouple and was maintained at 30 ± 0.5°C.

Experimental Protocol of Intact Preparations

Measured parameters of CaT and tension in control and DM myocardium. The magnitude of CaT converted to [Ca²⁺] (peak [Ca²⁺]), the time for aequorin light to reach its peak from the onset of stimulus (time to peak light, TPL), the time for aequorin light to decay from 75 to 25% of the peak (decay time), the time measured from the onset of stimulus to the peak (time to peak tension, TPT), and the time for tension to decrease from the peak to 50% (relaxation time) were measured in control and DM myocardium.

Tension-dependent changes in decay time of CaT and relaxation time of contraction in control and DM myocardium. Peak tension was varied by changing extracellular Ca²⁺ concentration ([Ca²⁺]₀; 1, 2, and 4 mM) and/or muscle length (84% L_max, 92% L_max, and L_max). For example, muscle length was altered from 84% L_max to L_max in the solution with 2 mM [Ca²⁺]₀. The muscle length change was repeated in the solutions containing 1 and 4 mM [Ca²⁺]₀. When the muscle length was changed during a regular stimulation, tension promptly changed but the peak of CaT did not change (rapid effect). We measured CaT and tension when both reached a new steady-state level (slow effect) ~10 min after length change (5, 16). Decay time of CaT and relaxation time of contraction were plotted against the relative peak tension normalized to the peak developed tension measured at 2 mM [Ca²⁺]₀. The regression lines were drawn. The slopes of the regression lines represent the dependence of decay time of CaT and relaxation time of contraction on relative peak twitch tension (13, 18). The slope is reported to represent a change in the Ca²⁺ affinity of TnC caused by cross-bridge attachment if Ca²⁺ removal function is constant (13, 18).

pCa-tension relation in aequorin-injected myocardium. Measurement of the pCa-tension relation in tetanic contraction was essentially similar to that reported previously (11, 19, 27, 31, 32). We measured [Ca²⁺] and tension during tetanic contraction when [Ca²⁺]₀ was altered from 0.25 to 16 mM. When the developed tension measured at 16 mM [Ca²⁺]₀ was larger than that at 8 mM [Ca²⁺]₀, [Ca²⁺]₀ was further increased up to 20 mM to produce maximal tension. During tetanic contraction, the aequorin light signal was measured through a 1-Hz low-pass filter to reduce noise. At low [Ca²⁺]₀, we induced six to seven contractions and three to five signals.

Table 1. Comparison of measured parameters of Ca²⁺ transients and tension in control and DM myocardium

<table>
<thead>
<tr>
<th></th>
<th>Peak [Ca²⁺], μM</th>
<th>TPL, ms</th>
<th>Decay Time, ms</th>
<th>Tension, mN/mm²</th>
<th>TPT, ms</th>
<th>Relaxation Time, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.55 ± 0.50</td>
<td>27.0 ± 2.68</td>
<td>29.8 ± 4.22</td>
<td>30.8 ± 12.8</td>
<td>112 ± 10</td>
<td>81 ± 12</td>
</tr>
<tr>
<td>DM</td>
<td>1.68 ± 0.45</td>
<td>29.9 ± 4.67*</td>
<td>36.8 ± 5.47*</td>
<td>32.9 ± 13.8</td>
<td>146 ± 18</td>
<td>128 ± 35</td>
</tr>
</tbody>
</table>

Values are means ± SD; nos. in parentheses are no. of observations. DM, diabetes mellitus; peak [Ca²⁺], magnitude of Ca²⁺ transient that was converted to intracellular Ca²⁺ concentration ([Ca²⁺]₀); TPL, time to peak light (time for aequorin light to reach peak from onset of stimulus); decay time, time for aequorin light to decay from 75 to 25% of peak; TPT, time to peak tension (time measured from onset of stimulus to peak); relaxation time, time for tension to decrease from peak to 50%. Values in DM preparations significantly different from those in control preparations: *P < 0.05, †P < 0.001.
were averaged to improve the signal-to-noise ratio. (Ca^{2+}; and tension, measured 6 s after the onset of the repetitive stimulation, were plotted and fitted using the Hill equation: 

\[ T = \frac{T_{\text{max}} \times [Ca^{2+}]^{n_{H}}}{[Ca^{2+}]^{n_{H}} + K_{d}^{n_{H}}} \]

where \( T \) is measured tension, \( T_{\text{max}} \) is maximal tension, \( K_{d} \) is the [Ca^{2+}; that produces 50\% of \( T_{\text{max}} \) (an indicator of the Ca^{2+}-sensitivity of the contractile elements), and \( n_{H} \) is the Hill coefficient (an indicator of the cooperativity of the myofilaments). We defined \( pCa \) as \(-\log[Ca^{2+}]\), and \( pCa_{0} \), as \(-\log K_{d}\).

Measurement of transient change in [Ca^{2+}] in a tetanic contraction. Six seconds after the onset of tetanic contraction, the muscle length was quickly (within 4 min) reduced from \( L_{\text{max}} \) to a shorter length (92, 88, and 84% \( L_{\text{max}} \)) using the electromagnetic motor to alter developed tension. The muscle length was restored for 2 s and then quickly (within 4 min) restored to \( L_{\text{max}} \) again. In response to the change in muscle length, a transient change in [Ca^{2+}] was observed (extra Ca^{2+}) (5, 13, 20, 31). To reduce noise, we used a 10-Hz low-pass filter to record the extra Ca^{2+}. The extra light signal was significantly slow that it was not affected by the filter. The magnitude of tension reduction was calculated by subtracting the developed tension measured 0.5 s after the length change from that measured immediately before the length change. Because the extra Ca^{2+} is known to be a function of the magnitude of tension reduction and [Ca^{2+}]; immediately before the length change, we plotted the magnitude of the extra Ca^{2+} normalized to the [Ca^{2+}]; immediately before length change against the magnitude of tension reduction, and regression lines were drawn for each group.

Measurement of cross-bridge cycling rate in a tetanic contraction. The cross-bridge cycling rate (CCR) was measured using a sinusoidal perturbation method (11, 32) in a tetanic contraction that was continued for ~30 s using a procedure reported previously (11, 19). When tetanic tension at 8 mM [Ca^{2+}]; reached steady-state level, sinusoidal length change <1% \( L_{\text{max}} \) of each preparation was applied using the electromagnetic motor. Determination of CCR was the same as in previous studies (11, 32).

Experiments With Skinned Preparations

Thin trabeculae (diameter, 0.1–0.2 mm) were dissected from the right ventricle of the same rats as those used for the experiments described in Experiments With Intact Preparations. The preparations were immersed in the relaxing solution containing 1% Triton X-100 for 60 min and washed with the relaxing solution without Triton X-100. The preparations were then immersed in the relaxing solution containing 50% glycerol and kept at \(-10°C\) before use. The mean cross-sectional area with the sarcomere length of 2.3 \( \mu \)m in DM (0.0113 \( \pm \) 0.00268 mm^2, \( n = 7 \)) was significantly smaller than that of the control (0.0241 \( \pm \) 0.00858 mm^2, \( n = 6 \)). The mean cross-sectional area in DM (0.00992 \( \pm \) 0.00189 mm^2, \( n = 5 \)) was also significantly smaller than that of the control (0.0236 \( \pm \) 0.00217 mm^2, \( n = 6 \)). The preparation was cut into small bundles (length 1–1.5 mm) in the relaxing solution and used for the experiments. Both ends of the preparation were tied with silk monofilaments, and the preparation was then carefully transferred to a muscle chamber of the same design as that reported by Horiuti (12). One end of the preparation was fixed to a tungsten wire (0.1-mm diameter) extending from the fixed arm, and the other end was attached to the arm of a tension transducer (BG-10, Kulite, Semiconductor Products). The sarcomere length of the preparation was adjusted to 2.3 \( \mu \)m by measuring the first order of laser diffraction lines, as previously reported (13).

Solutions and procedures for skinned preparations. The composition of the relaxing solution was (in mM) 88.6 potassium methanesulfonate, 4.5 ATP, 5.2 magnesium methanesulfonate, 10 EGTA, 20 PIPES, and 0.5 dithiothreitol, with 10 IU/ml creatine phosphokinase. pH was adjusted to 7.1 with KOH at 20°C. Free Ca^{2+}; concentration of the solution was calculated using the binding constant of each ion for each ligand (25). The calculated apparent dissociation constant of EGTA for Ca^{2+} was 407 nM. The concentrations of free Mg^{2+} and Mg-ATP were kept at 1.0 and 3.5 mM, respectively. The ionic strength was maintained at 0.2 M. The solutions with various pCa (\(-\log[Ca^{2+}]\)) were made by mixing the relaxing solution and the solution at a pCa of 4.0. The temperature of the solution was kept at 20 \( \pm \) 0.5°C throughout the experiment. The pCa-tension relation in each preparation was fitted by nonlinear least-squares regression to a Hill equation as described in pCa-tension relation in aequorin-injected myocardium.

Chemicals

STZ was purchased from Sigma Chemical (St. Louis, MO). Aequorin was purchased from Dr. J. R. Blinks (Friday Harbor, WA). dl-Butranol HCl was a gift from Kaken Pharmaceutical (Tokyo, Japan), and a 2 mM stock solution prepared by dissolving it in double-distilled water was stored at 0°C. Ryanodine was purchased from AgriSystem (Wind Gap, PA), and a 1 mM stock solution prepared by dissolving it in warmed double-distilled water was stored at 0°C. Na_{2}ATP was purchased from Boehringer Mannheim (Mannheim, Germany). EGTA was purchased from Wako Pure Chemical Industries (Tokyo, Japan). PIPES and phosphocreatine disodium salt were purchased from Nakarai Tesque (Kyoto, Japan). Methylene sulfonic acid and calcium methanesulfonate were from Tokyo Kasei (Tokyo, Japan). Creatine phosphokinase and dithiothreitol were from Sigma Chemical.

Statistics

The measured values are expressed as means \( \pm \) SD. Unpaired Student's t-test was used, and statistical significance was verified at \( P < 0.05 \). Correlations between developed tension and decay time of CaT and relaxation time of contraction were evaluated by testing the correlation of these parameters.

RESULTS

General Characteristics of STZ-Induced Diabetic Rats

Rats were used 4–7 wk after a single tail vein injection of STZ (50 mg/kg) for the DM group. The body weight (BW, g), wet weight (HW; mg), and HW-to-BW ratio (HW/BW) of the DM group (233 \( \pm \) 44, 880 \( \pm \) 195, and 3.81 \( \pm \) 0.41, respectively) were all significantly different from those of the control group (425 \( \pm \) 55, 1,346 \( \pm \) 248, and 3.16 \( \pm \) 0.31; \( n = 40 \)) (significant change: \( P < 0.001 \) for BW, \( P < 0.001 \) for HW, \( P < 0.001 \) for HW/BW). Serum blood glucose (mg/dl) in DM was 529 \( \pm \) 66 (\( n = 45 \)), which was significantly higher than that in control (217 \( \pm \) 72 (\( n = 39 \)), \( P < 0.001 \)). These changes verified that DM was effectively induced by the treatment.

Ca^{2+} Transients and Tension in Control and in DM Myocardium

Figure 1 shows CaT (fast signal) and tension (slow signal) in control myocardium (representative record from 22 experiments) and DM myocardium (representa-
Fig. 1. Ca2+ transients (CaT) and tension in control (A) and in diabetes mellitus (DM, B) myocardium. Representative records of CaT (aequorin light, faster signals) and tension (slower signals) measured at 2 mM extracellular Ca2+ concentration ([Ca2+]o) and length at which developed tension reached maximum (Lmax) are shown. Peaks of intracellular Ca2+ concentration ([Ca2+]i) and developed tension did not significantly differ in the 2 groups (see Table 1). To observe differences in time courses of tension and CaT, peaks of tension and CaT were normalized to match their peaks and superimposed (C and D, respectively). Time to peak tension and relaxation time were significantly prolonged in DM (see Table 1). Time to peak light and decay time were significantly prolonged in DM (see Table 1).

The length-tension relation that reflects the intrinsic length-dependent activation process in mammalian

Tension-Dependent Changes in Decay Time of CaT and Relaxation Time of Contraction in Control and DM Myocardium

To investigate the tension-dependent changes in decay time of CaT and relaxation time of contraction in both groups, which represent the change in the Ca2+ affinity of TnC caused by active cross bridges (see Refs. 5, 13, and 18 for details), CaT and tension were measured when developed tension was varied by changing [Ca2+]o in the solution and/or the muscle length. Figure 2 shows CaT and tension measured at different muscle lengths in the solution with 2 mM [Ca2+]o in DM myocardium (representative record from 10 experiments) at 2 mM [Ca2+]o and Lmax. The measured parameters in twitch contraction are summarized in Table 1.

The magnitude of developed tension in DM myocardium (32.9 ± 13.8 mN/mm2, n = 27) was not significantly different from that in control myocardium (30.8 ± 12.8 mN/mm2, n = 22) (Fig. 1, A and B). However, the time courses of contraction (TPL and relaxation time of contraction) in DM myocardium were significantly prolonged (146 ± 18 and 128 ± 35 ms, respectively; n = 27) compared with those in control myocardium (112 ± 10 and 81 ± 12 ms, respectively; n = 22) (P < 0.001 for TPT, P < 0.001 for relaxation time of contraction; Fig. 1C).

Peak [Ca2+]i in DM myocardium (1.68 ± 0.45 µM, n = 19) was not significantly different from that in control myocardium (1.55 ± 0.50 µM, n = 20; Fig. 1, A and B). TPL was slightly but significantly prolonged in DM myocardium (29.9 ± 4.67 ms, n = 19) compared with that in control myocardium (27.0 ± 2.68 ms, n = 20) (P < 0.05; Fig. 1D). Decay time of CaT in DM myocardium was significantly prolonged (36.8 ± 5.47 ms, n = 19) compared with that in control myocardium (29.8 ± 4.22 ms, n = 20) (P < 0.001; Fig. 1D).

Tension-Dependent Changes in Decay Time of CaT and Relaxation Time of Contraction in Control and DM Myocardium

To observe differences in time courses of tension and CaT, peaks of tension and CaT were normalized to match their peaks and superimposed (C and D, respectively). Time to peak tension and relaxation time were significantly prolonged in DM (see Table 1). Time to peak light and decay time were significantly prolonged in DM (see Table 1).

Correlation between decay time of CaT and relative peak tension in control and DM myocardium was quantitatively identical to those in ferret myocardium in our previous studies (13, 18), which indicates that the dependence of decay time of CaT and relaxation time of contraction on relative tension reflects the tension-dependent change in the affinity of TnC for Ca2+ (5, 13, 16, 18, 20). However, both lines in DM myocardium were altered to the vertical and upward directions compared with those in control myocardium. The absolute values of the slopes of decay time of CaT and relaxation time of contraction lines in DM were significantly higher than those in the control (P < 0.001 for decay time of CaT, P < 0.001 for relaxation time of contraction).
Decay time of CaT and relaxation time of contraction were significantly larger than those in control (\(P < 0.001\)) in DM myocardium. Absolute value of slopes of the 2 regression lines in DM was significantly dependent on relative peak tension in control and DM myocardium. The value of \(n\) in DM myocardium (3.17 \pm 0.90, \(n = 10\)) was not significantly different from that in control myocardium (2.8 \pm 0.76, \(n = 10\)).

Cardiac muscles was measured in both groups at 2 mM \([\text{Ca}^{2+}]_o\). Because \([\text{Ca}^{2+}]_o\) substantially influences length-tension relation, \([\text{Ca}^{2+}]_o\) was fixed at 2 mM (3). In accordance with the shortening of muscle length from \(L_{\text{max}}\) to 92% \(L_{\text{max}}\) or to 84% \(L_{\text{max}}\), developed tension was significantly decreased in both groups. The length-tension relation in cardiac muscles measured immediately after muscle length change differed from that measured at a steady state several minutes after the alteration in muscle length (4, 5). Our results were obtained when the developed tension reached a steady state. The magnitude of the developed tension at 84% \(L_{\text{max}}\) (6.9 \pm 7.2 mN/mm\(^2\), \(n = 11\)), 92% \(L_{\text{max}}\) (15.9 \pm 12.2 mN/mm\(^2\), \(n = 11\)), and \(L_{\text{max}}\) (30.2 \pm 15.2 mN/mm\(^2\), \(n = 11\)) in DM myocardium did not significantly differ from that of the control myocardium (4.1 \pm 2.8 mN/mm\(^2\), \(n = 8\)) at 84% \(L_{\text{max}}\) 10.7 \pm 7.5 mN/mm\(^2\), \(n = 9\) at 92% \(L_{\text{max}}\), and 26.7 \pm 11.2 mN/mm\(^2\), \(n = 10\) at \(L_{\text{max}}\). Therefore, the length-dependent change in the developed tension in twitching isolated myocardium was similar in both groups for a muscle length that changes under physiological conditions.

**Fig. 3.** Tension-dependent changes in decay time of CaT (A) and relaxation time of contraction (B) in control (○) and DM (●) myocardium. Peak tension was altered as described in MATERIALS AND METHODS. Decay time of CaT and relaxation time of contraction were plotted against relative peak tension (tension (relative)) (each measured value was normalized to that measured at 2 mM \([\text{Ca}^{2+}]_o\), and \(L_{\text{max}}\) in each preparation). Regression lines were drawn; equations, correlation coefficients, and levels of significance are as follows. A: control, \(Y = -2.58X + 35.3\) (\(R = -0.48, P < 0.002\)); DM, \(Y = -9.13X + 48.2\) (\(R = -0.59, P < 0.001\)). B: control, \(Y = 14.0X + 56.9\) (\(R = 0.70, P < 0.001\)); DM, \(Y = 36.8X + 76.2\) (\(R = 0.61, P < 0.001\)).

Both decay time of CaT and relaxation time of contraction were significantly dependent on relative peak tension in control and DM myocardium. Absolute value of slopes of the 2 regression lines in DM were significantly larger than those in control (\(P < 0.001\) in A and \(P < 0.001\) in B).
Thus none of the parameters measured in the pCa-tension relation in intact DM myocardium were significantly different from those in control myocardium.

Extra Ca\(^{2+}\) Measured in Aequorin-Injected Tetanic Contraction in Control and DM Myocardium

To further investigate whether the tension-dependent change in the Ca\(^{2+}\) affinity of TnC in DM myocardium is different from that in control myocardium, we measured the extra Ca\(^{2+}\) that was induced by a quick tension change during a tetanic contraction (Fig. 5A). Muscle length was quickly shortened 6 s after the onset of the repetitive stimulation, because the pCa-tension relation at 6 s after stimulation in both groups was essentially identical (Table 2). In accordance with the quick shortening of the muscle length, developed tension was quickly decreased and extra Ca\(^{2+}\) (Fig. 5A) was produced, which reached the maximal level ~100–150 ms after the commencement of the quick release. Tension then rapidly recovered to a slight extent. According to Saeki et al. (31), the time course of the decay of the extra Ca\(^{2+}\) corresponds to the time course of the redeveloped tension. However, this was not clearly observed in the present experiment. This might be caused by the relatively faster change in tension in rat myocardium than that in ferret myocardium and might be partly caused by the filtered extra Ca\(^{2+}\) signal.

The normalized magnitude of the extra Ca\(^{2+}\) in DM and control myocardium (data points obtained from 4 experiments in DM and 3 experiments in control) was plotted against the magnitude of tension reduction, and regression lines were drawn (Fig. 5B). The highly significant correlation coefficients indicate that the normalized extra Ca\(^{2+}\) is dependent on the magnitude of tension reduction in both groups (13, 20). However, the regression lines were not significantly different, which suggests that the amount of Ca\(^{2+}\) dissociated from the Tn-Ca\(^{2+}\) complex as a result of the same amount of tension change is essentially identical in control and DM myocardium and that the tension-dependent feedback mechanism in DM myocardium functions the same as in control myocardium. Thus the data regarding length-tension relation, pCa-tension relation (Fig. 4 and Table 2), and normalized extra Ca\(^{2+}\) (Fig. 5B) support our view that the tension-dependent change in the Ca\(^{2+}\) affinity of TnC functions similarly in control and DM intact myocardium. Therefore, more remarkable tension dependence of decay time of CaT

Table 2. Comparison of measured parameters of pCa-tension relations in intact and skinned preparations in control and DM myocardium

<table>
<thead>
<tr>
<th>Length</th>
<th>Intact Preparations (L_{max})</th>
<th>Skinned Preparations</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SL = 7.8 μm</td>
<td>SL = 1.9 μm</td>
</tr>
<tr>
<td></td>
<td>Control (9)</td>
<td>DM (10)</td>
</tr>
<tr>
<td>pCa_{50}</td>
<td>6.22 ± 0.66</td>
<td>6.14 ± 0.48</td>
</tr>
<tr>
<td>n_{H}</td>
<td>3.46 ± 1.1</td>
<td>3.17 ± 0.90</td>
</tr>
<tr>
<td>Maximal tension</td>
<td>42.8 ± 7.8</td>
<td>41.8 ± 9.7</td>
</tr>
</tbody>
</table>

Values are means ± SD; nos. in parentheses indicate no. of observations. pCa_{50}, − log of [Ca\(^{2+}\)]_0; n_{H}, Hill coefficient; L_{max}, length at which developed tension reached maximum in intact preparations; SL, sarcomere length measured by laser diffraction lines in skinned preparations. *P < 0.05 compared with control.
before, the Ca$^{2+}$ sensitivities of the contractile elements at different sarcomere lengths in both groups were essentially identical. The value of $n_\text{H}$ at 2.3 µm in DM myocardium (5.28 ± 0.89, n = 7) was significantly increased compared with that in control myocardium (4.28 ± 0.29, n = 6) (P < 0.05). However, at 1.9 µm, the value of $n_\text{H}$ in DM myocardium (5.52 ± 0.61, n = 5) was identical with that in control myocardium (5.52 ± 0.53, n = 6). $T_\text{max}$ measured at 2.3 µm (74.5 ± 16.9 mN/mm$^2$, n = 7) and 1.9 µm (59.8 ± 29.3 mN/mm$^2$, n = 5) in DM was not significantly different from that in control [83.5 ± 43.3 mN/mm$^2$ (n = 6) at 2.3 µm and 50.8 ± 17.7 mN/mm$^2$ (n = 6) at 1.9 µm]. Therefore, all of the parameters measured in the pCa-tension relation in DM myocardium were not significantly different from those in control myocardium, except for a slightly but significantly increased $n_\text{H}$ at 2.3 µm in DM (Table 2). Thus the properties of the myofilaments were relatively similar in both control and DM myocardium. We then measured CCR in both groups, which is related to the time course of contraction.

CCR Measured Using Sinusoidal Perturbation Method in Control and DM Myocardium

Figure 7A shows a representative record of tension responses to a small sinusoidal length change in DM preparations (from 8 experiments). Concomitant with the increase in frequency, the tension in response to the sinusoidal length changes became smaller and reached the minimal frequency (dip) (11, 30, 32). If the frequency was increased beyond the dip frequency, tension response became larger again. The CCR in DM myocardium was significantly slower (2.7 ± 0.3 Hz, n = 8) than that in control myocardium (4.7 ± 0.5 Hz, n = 6) (P < 0.001; Fig. 7B).

**Fig. 5.** Effects of a quick muscle length change on Ca$^{2+}$ signal and tension in a control preparation (A). Top trace, muscle length. Second trace, [Ca$^{2+}$]. Arrow indicates extra Ca$^{2+}$. Third trace, tension. Fourth trace, difference in tension at L$_{\text{max}}$ and 88% L$_{\text{max}}$ (tension reduction). Bottom trace, difference in [Ca$^{2+}$] measured with and without tension reduction (extra Ca$^{2+}$). B: relation between magnitude of extra Ca$^{2+}$ and tension reduction in control (○) and DM (●) myocardium. Magnitude of extra Ca$^{2+}$ normalized to [Ca$^{2+}$]$_i$ immediately before length change (extra Ca$^{2+}$/[Ca$^{2+}$]$_i$) (ordinate) was plotted against magnitude of tension reduction. These experiments were carried out in solution with 16 mM [Ca$^{2+}$]$_o$. Magnitude of tension reduction was normalized to developed tension at L$_{\text{max}}$ (tension reduction) (abscissa). Equations, correlation coefficients, and levels of significance are as follows. Control, Y = 0.00426X + 0.0164 (R = 0.860, P < 0.001). DM, Y = 0.00392X + 0.0147 (R = 0.997, P < 0.001). Normalized extra Ca$^{2+}$ showed a highly significant dependence on tension reduction in each preparation. Slopes of the 2 regression lines in control and DM were not statistically different.

**Fig. 6.** pCa-tension relation in skinned preparations in control (○) and DM (●) myocardium at long (2.3 µm) and in control (△) and DM (▲) myocardium at short (1.9 µm) sarcomere lengths. Curves are normalized and fitted with Hill equation. Tension at each pCa is expressed relative to maximum tension obtained at pCa 4.95. pCa$_{50}$ values at both sarcomere lengths in DM myocardium were similar to those in control myocardium. Hill coefficient in DM myocardium at sarcomere length of 2.3 µm was slightly but significantly larger than that in control myocardium of same sarcomere length (P < 0.05; see Table 2).
**Slower Contraction in DM Myocardium**

Slower contraction in DM myocardium is caused by slower Ca\(^{2+}\) handling in SR (slower Ca\(^{2+}\) release and slower Ca\(^{2+}\) uptake) as discussed in Alterations in Ca\(^{2+}\) in DM Myocardium and is caused by the slower CCR (Fig. 7). The slower CCR is related to the alteration of myosin isoform from V1 to V3 (30) that is consistently reported in STZ-induced DM rat myocardium (8, 33). Thus the slower time course of tension measured as shortening or tension. Some reports support our results: developed tension in DM and control myocardium (9, 23, 33) and the magnitude of CaT and shortening in DM and control myocytes (34) do not significantly differ.

The slower decay time of CaT in DM myocardium could be explained by the decreased activity of the Ca\(^{2+}\)-removal mechanisms [SR (22, 28), Na\(^+\)/Ca\(^{2+}\) exchanger, and sarcotemmal Ca\(^{2+}\) pump (24)] and the lower affinity of TnC for Ca\(^{2+}\) (a major Ca\(^{2+}\) buffer in the myoplasm). In rat ventricular muscles, however, SR plays a pivotal role for Ca\(^{2+}\) sequestration, and the contribution of the Na\(^+\)/Ca\(^{2+}\) exchanger is <10% (7). Therefore, the slower decay of CaT is mainly caused by the slower Ca\(^{2+}\) uptake by SR if the affinity of TnC for Ca\(^{2+}\) in DM myocardium is not significantly altered. Although it is reported that the relative contribution of the Na\(^+\)/Ca\(^{2+}\) exchanger is slightly increased in DM (22), this would not explain the slower CaT. A similar decay of CaT in thapsigargin-treated control and DM myocytes supports the view that the slower Ca\(^{2+}\) uptake by SR in DM myocardium is a major factor for the slower CaT (22). In addition, the prolonged action potential duration caused by a reduction in the transient outward current (15) without accompanying a change in L-type Ca\(^{2+}\) current density (15, 34) in DM myocytes might be a factor in the slower time course of CaT. However, a significant change in the action potential duration is not recognized in papillary muscles at the early stage of STZ treatment (6). Thus the duration of action potential might not be a factor to explain the slower CaT.

The slightly but significantly slower time to peak of CaT in DM myocardium compared with that in control myocardium (Table 1, Fig. 1D) might be caused by the slower Ca\(^{2+}\) release from SR. A decrease in the number of ryanodine-sensitive receptors in SR (36) without a change in L-type Ca\(^{2+}\) channels suggests an alteration in the Ca\(^{2+}\)-release channels, which might explain the slower time to peak of CaT.

Slower Contraction in DM Myocardium

Slower contraction in DM myocardium is caused by slower Ca\(^{2+}\) handling in SR (slower Ca\(^{2+}\) release and slower Ca\(^{2+}\) uptake) as discussed in Alterations in Ca\(^{2+}\) in DM Myocardium and is caused by the slower CCR (Fig. 7). The slower CCR is related to the alteration of myosin isoform from V1 to V3 (30) that is consistently reported in STZ-induced DM rat myocardium (8, 33). Thus the slower time course of tension in DM myocardium could be explained by the dysfunction of the Ca\(^{2+}\)-uptake mechanism in SR and the slower CCR. However, because the alteration in the affinity of TnC for Ca\(^{2+}\) also influences relaxation time of contraction, we should consider this possibility as a reason for the slower time to peak of CaT.
course of contraction. It is reported that an increase in the affinity of TnC for Ca\(^{2+}\) retards relaxation and inversely shortens the decay time of CaT (5, 13, 18). Therefore, the change in the affinity of TnC for Ca\(^{2+}\) in DM myocardium is a possible factor to explain the slower relaxation time of contraction and the slower decay time of CaT. This is discussed below.

pCa-Tension Relation in Intact and Skinned Preparations in DM

The quantitative different pCa-tension relations measured in intact and skinned preparations (Figs. 4 and 6) have already been reported, and the reasons for the difference have been discussed (19). We assumed that the intracellular ionic condition was not significantly different in DM and control myocardium, and we measured the pCa-tension relation using tetanic contraction. pCa-tension relations of tetanic contraction in the two groups were not significantly different, as shown in Fig. 4. In addition, pCa-tension relations in skinned preparations in DM and control myocardium were also identical (Fig. 6). Therefore, the contractile element in DM myocardium responded to Ca\(^{2+}\) just as that in control myocardium at steady state, although differing results have been reported (1, 10, 17). The reason for the different results is not clear at present.

In addition, pCa-tension relations measured at different muscle lengths in DM were similar to those in control myocardium (Fig. 6). Therefore, a change in muscle length shows a similar effect on the affinity of TnC for Ca\(^{2+}\) in both groups, which is consistent with the results regarding the extra Ca\(^{2+}\) produced by a quick muscle length change during tetanic contraction (Fig. 5).

The results of the present study (Figs. 4 and 6) also demonstrated that the cooperativity of both groups shown as the Hill coefficient was similar. Thus, at a steady state, the mechanism of the tension-dependent change in the affinity of TnC for Ca\(^{2+}\) in DM myocardium is considered to work the same as in control myocardium.

Tension-Dependent Change in Affinity of TnC for Ca\(^{2+}\) in DM Myocardium

The tension-dependent change in the affinity of TnC for Ca\(^{2+}\) during contraction in DM myocardium is an interesting issue to explore. A quick reduction of muscle length (tension reduction) decreases the affinity of TnC for Ca\(^{2+}\), and the Ca\(^{2+}\) dissociated from the TnC-Ca\(^{2+}\) complex appears as the extra Ca\(^{2+}\) (Fig. 5A). The increased Ca\(^{2+}\) decays during tension reduction and the decrease in the extra Ca\(^{2+}\) might be caused by Ca\(^{2+}\)-removal mechanisms, in particular because of the Na\(^{+}\)/Ca\(^{2+}\) exchanger (SR is already blocked by ryanodine in tetanic contraction) and/or rebinding of Ca\(^{2+}\) to TnC. The normalized peak of the extra Ca\(^{2+}\) in both groups did not significantly differ (Fig. 5B). Therefore, tension-dependent change in the affinity of TnC for Ca\(^{2+}\) is working during contraction in DM myocardium similarly as in control myocardium. This result supports the view that the different dependence of relaxation time of contraction and decay time of CaT on tension (Fig. 3) cannot be explained by the tension-dependent change in the affinity of TnC for Ca\(^{2+}\).

Contribution of SR to Dependence of Decay Time of CaT and Relaxation Time of Contraction on Developed Tension

The decay time of CaT shortens as the peak of twitch tension increases (Fig. 3A), which is considered to reflect tension-dependent change in the affinity of TnC for Ca\(^{2+}\) (5, 13, 18, 20). The dependence of decay time of CaT on peak tension is also influenced by the rate of Ca\(^{2+}\) uptake by SR as well as the change in the affinity of TnC for Ca\(^{2+}\): a faster Ca\(^{2+}\) uptake by SR offsets the change in decay time of CaT (13). In contrast, relaxation time of contraction increases as a function of peak twitch tension, which is caused by the higher Ca\(^{2+}\) affinity of TnC. In DM myocardium, however, the dependence of decay time of CaT and relaxation time of contraction on peak twitch tension is larger compared with that in control myocardium (Fig. 3). There are two possibilities to explain the larger dependence of decay time of CaT and relaxation time of contraction on peak twitch tension in DM myocardium, a larger tension-dependent change in the affinity of TnC for Ca\(^{2+}\) and/or slower Ca\(^{2+}\) uptake by SR. Because the tension-dependent change in the affinity of TnC for Ca\(^{2+}\) in DM myocardium did not differ from that in control myocardium (Fig. 5), the slower Ca\(^{2+}\) uptake by SR is the most likely factor to explain the larger dependence of decay time of CaT on tension in DM myocardium, which is opposite to that in hyperthyroid myocardium (13). Therefore, Ca\(^{2+}\)-removal activity substantially modulates the apparent dependence of decay time of CaT and relaxation time of contraction on developed tension.

In conclusion, the most significant characteristic of DM myocardium is the slower time courses of CaT and contraction; the contractile element of DM myocardium responded to Ca\(^{2+}\) just as in control myocardium. In addition, the tension-dependent change in the affinity of TnC for Ca\(^{2+}\) in DM myocardium functions the same as in control myocardium. The slower CCR and the slower Ca\(^{2+}\) uptake by SR are possible factors to explain the slower CaT and contraction in DM myocardium. The apparent larger dependence of decay time of CaT and relaxation time of contraction is attributable to the slower Ca\(^{2+}\) uptake rate by the sarcoplasmic reticulum.

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