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Dantrolene: effects on abnormal intracellular Ca^{2+} handling and inotropy in postinfarcted rat myocardium

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Abstract

The present study was designed to investigate the effects of dantrolene on intracellular Ca^{2+} ($[Ca^{2+}]_i$) handling and inotropy in rat infarcted myocardium. Dantrolene-treated rats with myocardial infarction were placed into two different dosage groups. The infarcted control group received placebo only. Isometric contractility and intracellular Ca^{2+} transients were recorded simultaneously in isolated papillary muscles. Diastolic $[Ca^{2+}]_i$ was significantly lower in muscle preparations from infarcted rats receiving dantrolene compared to the placebo control group. Additionally, treatment with dantrolene in infarcted rats significantly improved the inotropic response to 10^{-4} M isoproterenol. The protein levels of the sarcoplasmic reticulum Ca^{2+} ATPase were increased in infarcted rat hearts with dantrolene treatment. We conclude that dantrolene improved the inotropic response to β-adrenoceptor stimulation in rat postinfarcted myocardium, which is related to improved intracellular Ca^{2+} handling, and lowered diastolic Ca^{2+} concentration.

Keywords: Dantrolene; Myocardial infarction; Ca^{2+}, intracellular; Myocardial contractility

1. Introduction

It has been demonstrated that intracellular Ca^{2+} ($[Ca^{2+}]_i$) plays a central role in the process of excitation–contraction coupling in the heart. Alterations in the subcellular handling of Ca^{2+} provide the basis for cardiac contractile failure (Morgan and Morgan, 1984; Morgan, 1991). Excitation–contraction coupling is initiated when depolarization permits Ca^{2+} to enter the myoplasm and subsequently induces release of a large quantity of activator Ca^{2+} from intracellular stores in the sarcoplasmic reticulum. The released Ca^{2+} interacts with troponin C and results in cardiac contraction. Relaxation occurs when Ca^{2+} dissociates from the contractile apparatus and is resequestered by the energy-dependent Ca^{2+} pump of the sarcoplasmic reticulum. Dysregulation of Ca^{2+} homeostasis may reflect impairment of sarcoplasmic reticulum function, which could decrease the Ca^{2+} resequestration and induce myoplasmic Ca^{2+} overload (Gwathmey et al., 1987). Morgan et al. (1990) reported that contractions and Ca^{2+} transients of failing cardiac muscles were markedly prolonged, and the aequorin Ca^{2+} transients exhibited two distinct components. Muscles from failing hearts showed a diminished capacity to restore a low resting Ca^{2+} level during diastole. These data suggest that intracellular Ca^{2+} handling is abnormal and causes both systolic and diastolic dysfunction in heart failure. Thus, therapeutic interventions modifying the abnormality of intracellular Ca^{2+} may produce potential beneficial contributions in heart failure.

Dantrolene sodium (1-[[[5-(4-nitrophenyl)-2-furanyl]methylene]amino]-2,4-imidazolidinedione sodium salt hydrate) acts primarily by affecting Ca^{2+} flux across the sarcoplasmic reticulum, and has been successfully used in the treatment of several rare hypercatabolic syndromes; e.g., malignant hyperthermia. The mechanism of malignant hyperthermia might be related to Ca^{2+}-induced Ca^{2+} release, resulting in an increase of the free ionized $[Ca^{2+}]_i$ to toxic levels, i.e., Ca^{2+} overload (Ellis and Heffron, 1985).

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In skeletal muscle, other reports showed that dantrolene inhibits Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum, subsequently ameliorating intracellular Ca^{2+} overload in malignant hyperthermia (Ohta et al., 1990; Lopez et al., 1992). A previous study (Meissner et al., 1996) with Ca^{2+}-overloaded rat cardiac muscles in vitro found that dantrolene increased the initial Ca^{2+} uptake rate by 23% and reduced the amplitude of Ca^{2+} oscillations, suggesting that dantrolene modifies Ca^{2+} handling by myocardial sarcoplasmic reticulum. It is reasonable to hypothesize that dantrolene is a clinically applicable drug with certain specific characteristics, i.e., modifies the impaired Ca^{2+} homeostasis and has a mild negative inotropic effect in cardiac muscle. To clarify this speculation, the present study was designed to investigate the effects of dantrolene on intracellular Ca^{2+} regulation and inotropic responsiveness to β-adrenoceptor stimulation in postinfarcted rat myocardium.

2. Materials and methods

2.1. Isometric contractility and intracellular Ca^{2+} measurement in infarcted rat myocardium

2.1.1. Drug randomization and myocardial infarction model

Male Lewis rats (Charles River) initially weighing ~ 250 g were used in the study. The investigation conformed to the guide for the *Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the protocol was approved by the Institutional Animal Care Committee. Myocardial infarction was induced by ligation of the left anterior descending coronary artery under anesthesia with pentobarbital (60 mg/kg) by intraperitoneal injection (i.p.) with a modified technique described previously (Min et al., 1999, 2000). The successful ligation was verified by surface electrocardiography and observed blanching of the myocardium distal to the ligation. The sham-operated rats underwent an identical surgical procedure without ligation of the coronary artery.

The animals were treated with dantrolene by intraperitoneal injection once daily for 7 days prior to induction of myocardial infarction, and continued until the animals were sacrificed at 6 weeks after infarction. Placebo-controlled infarcted rats were treated with the same volume of distilled water by intraperitoneal injection, and sacrificed at 6 weeks after surgical procedure. Dantrolene sodium, a kind donation by Procter and Gamble Pharmaceuticals Deutschland, was dissolved in distilled water at different concentrations. All rats were placed into one of the following groups: Sham, sham-operated rats with neither ligation of the coronary artery nor drug treatment; Placebo-MI, placebo-treated rats with coronary ligation; Dantrolene-I, infarcted rats with daily intraperitoneal injection of 2 mg/kg dantrolene; Dantrolene-II, infarcted rats with daily intraperitoneal injection of 5 mg/kg dantrolene. The doses of dantrolene were chosen on the basis of previous reports (Wara et al., 1986; Ohkusa et al., 1998), and were adjusted according to body weight measured on the first day of every week after myocardial infarction. Each group consisted of eight rats.

2.1.2. Isometric muscle performance and aequorin light signal measurement

Six weeks after surgery, the animals were anesthetized with pentobarbital to measure blood pressure. The carotid artery was isolated and cannulated with a 3-Fr high-fidelity microtip catheter connected to a pressure transducer (Millar Instruments, Houston, TX, USA). The Millar Mikro-Tip catheter was advanced into the artery to record blood pressure and heart rate in a chart-strip recorder (model 56-1X 40-006158, Gould Instrument Systems, Cleveland, OH, USA) and averaged over a 10-min period. After measurement, the heart was rapidly excised and placed in a dissecting chamber containing modified Krebs–Henseleit solution with the following composition (in mM): 120 NaCl, 5.9 KCl, 5.5 dextrose, 25 $NaHCO_3$, 1.2 NaH_2PO_4, 1.2 $MgCl_2$ and 1.0 $CaCl_2$ (pH 7.4), bubbled with carbogen at room temperature. The posterior left ventricular papillary muscle was dissected and isometric contraction of the muscle was evaluated (Min et al., 1999, 2000). Briefly, the isolated papillary muscle was fixed to a muscle holder with a spring clip. The tendinous end of the muscle was vertically connected to a strain-gauge tension transducer with a silk thread. The muscle was then mounted in a 50-ml tissue bath containing modified Krebs–Henseleit solution maintained at 30 °C and continuously bubbled with carbogen. The isometric contraction of the papillary muscle was elicited by a punctate platinum electrode with square-wave pulses of 5-ms duration at 0.33 Hz. The voltage was set to 10% above threshold level. After 30-min equilibration period, the muscle was carefully stretched to the length at which maximal tension occurred. The following isometric contraction parameters were recorded from each muscle: developed tension (tension produced by the stimulated muscle), time to peak tension (time from the beginning of the contraction to peak tension) and time to 50% relaxation (time from peak tension to 50% relaxation). Subsequently, the loading procedure of aequorin was performed (see below). At the end of the experiment, the muscle was blotted and weighed. The cross-sectional area was determined from muscle weight and length by assuming a uniform cross section and a specific gravity of 1.05. After removal of the papillary muscle for studying, the weights of the right and left ventricles (including the septum) were normalized by body weight and used as indices of hypertrophy.

Aequorin (Dr. John Blinks, Friday Harbor Lab., San Juan Island, WA, USA) was loaded by the macroinjection technique (Min et al., 1999, 2000). Analog signals from the isometric force transducer and electronic photometer were recorded with a chart-strip recorder (Model 56-1X 40-

Table 1
General characteristics of sham-operated and infarcted rats with or without dantrolene treatment

	Sham	Placebo-MI	Dantrolene-I	Dantrolene-II
BW (g)	423 ± 25	408 ± 16	409 ± 15	414 ± 17
LVW (mg)	749 ± 54	1005 ± 53[a]	1012 ± 48[a]	786 ± 34
RVW (mg)	273 ± 41	278 ± 58	281 ± 39	264 ± 37
LVW/BW (mg/g)	1.78 ± 0.07	2.55 ± 0.15[a]	2.43 ± 0.19[a]	1.83 ± 0.16
RVW/BW (mg/g)	0.65 ± 0.12	0.69 ± 0.16	0.72 ± 0.16	0.68 ± 0.14
CSA (mm^2)	0.71 ± 0.07	0.92 ± 0.09[a]	0.93 ± 0.10[a]	0.70 ± 0.08
HR (beats/min)	396 ± 15	404 ± 13	386 ± 9	392 ± 10
MAP (mm Hg)	108 ± 13	78 ± 8[a]	80 ± 10[a]	94 ± 11

Values are means ± S.D. BW, body weight; LVW, left ventricular weight; RVW, right ventricular weight; LVW/BW, ratio of left ventricular weight/body weight; RVW/BW, ratio of right ventricular weight/body weight; CSA, muscle cross-sectional area; HR, heart rate; MAP, mean arterial blood pressure.

[a] $P<0.05$ vs. Sham and Dantrolene-II.

006158, Gould Instrument Systems). Signals including the amplitude of the light transient, time to peak light and time from peak to 50% decline in light transient were collected for further analysis. The free intracellular concentration of calcium ($[Ca^{2+}]_i$) was estimated by normalizing the recorded light signal during the isometric twitch by the maximal amount of light emitted after lysis of the muscle membranes at the end of the experiment with a 5% solution of the detergent, Triton X-100, in phosphate-free physiological salt solution containing 50 mM Ca^{2+} (Min et al., 1999, 2000). After obtaining baseline parameters, isoproterenol (10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) was added cumulatively to determine the inotropic response to β-adrenoceptor stimulation. Light signal and isometric contraction were measured at the plateau of the inotropic response to each addition of isoproterenol that was reached after 10 min.

2.1.3. Western blot analysis

The protein levels of sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2) and phospholamban were measured in additional left ventricles (five for each group) from sham-operated rats, placebo-treated rats 6 weeks after infarction, low-dose (2 mg/kg, i.p.) dantrolene-treated rats 6 weeks after infarction, and high-dose (5 mg/kg, i.p.) dantrolene-treated rats 6 weeks after infarction. Briefly, samples were homogenized at 4 °C in four volumes of 150 mmol/l NaCl. Protein concentrations were determined with a modified Bradford reaction (Bio-Rad, Hercules, CA, USA) using bovine serum albumin as a standard. Equal amounts of total protein (100 μg/lane) were electrophoresed and separated on a 10% Pro-cast gel (Novex Electrophoreses, San Diego, CA, USA). Separated proteins were transferred to nitrocellulose membranes blocked in 5% (w/v) nonfat dry milk in phosphate-buffered saline. After the membrane was rinsed, it was separately incubated overnight with a primary single antibody (SERCA2 anti-mouse monoclonal antibody or phospholamban anti-mouse monoclonal antibody, 1:1000 dilution, Affinity Bioregents, Golden, CO, USA). After being rinsed in phosphate-buffered saline, the membrane was incubated with peroxidase-labeled mouse antibodies to immunoglobulin G (IgG). Antibody reactions were developed with an enhanced chemiluminescence detection system (ESL, Amersham, Buckinghamshire, UK) and exposed to Kodak MR film for 40–60 s. The relative binding of the antibody to the SERCA2 and phospholamban were determined densitometrically using the NIH imaging system.

2.2. Statistical analysis

All data are given as means ± S.D. Differences between groups were evaluated by one-way analysis of variance (ANOVA). Means shown to be different between individual groups were compared using paired or unpaired Student's *t*-test and were considered significant at $P<0.05$.

3. Results

Six weeks after myocardial infarction, the ratio of left ventricular weight to body weight and the papillary muscle cross-sectional area significantly increased in the placebo-treated group and the Dantrolene-I group receiving 2 mg/kg dantrolene with intraperitoneal injection. However, cardiac

Table 2
Mechanical performance and intracellular Ca^{2+} concentration in papillary muscles isolated from sham-operated rats and postinfarcted rats with or without dantrolene treatment

	DT (mN/mm^2)	TPT (ms)	RT_{50} (ms)	TPL (ms)	RL_{50} (ms)	Syst. $[Ca^{2+}]_i$ (μM)	Diastolic $[Ca^{2+}]_i$ (μM)
Sham	12.8 ± 1.5	87.3 ± 8.4	55.3 ± 4.5	38.2 ± 2.8	44.8 ± 4.1[a]	0.61 ± 0.07	0.28 ± 0.03[a]
Placebo-MI	11.7 ± 1.9	103.4 ± 9.5[b]	59.5 ± 4.2[b]	45.7 ± 4.28[b]	51.2 ± 4.2[b]	0.60 ± 0.09	0.35 ± 0.05[b]
Dantrolene-I	11.3 ± 1.2	115.1 ± 7.6[b]	60.1 ± 5.0[b]	50.4 ± 3.7[c]	49.6 ± 5.3[b]	0.58 ± 0.05	0.34 ± 0.04[b]
Dantrolene-II	10.7 ± 1.4	112.4 ± 9.2[b]	62.3 ± 5.7[b]	51.2 ± 3.9[c]	45.3 ± 3.2[a]	0.56 ± 0.04	0.29 ± 0.02[a]

Values are means ± S.D. DT, developed tension; TPT, time to peak tension; RT_{50}, time from peak tension to 50% relaxation; TPL, time to peak light signal; RL_{50}, time from peak light to 50% decline; Syst. $[Ca^{2+}]_i$, peak systolic free intracellular Ca^{2+} concentration; Diastolic $[Ca^{2+}]_i$, diastolic free intracellular Ca^{2+} concentration.

[a] $P<0.05$ vs. Placebo-MI.

[b] $P<0.05$ vs. Sham.

[c] $P<0.01$ vs. Sham.

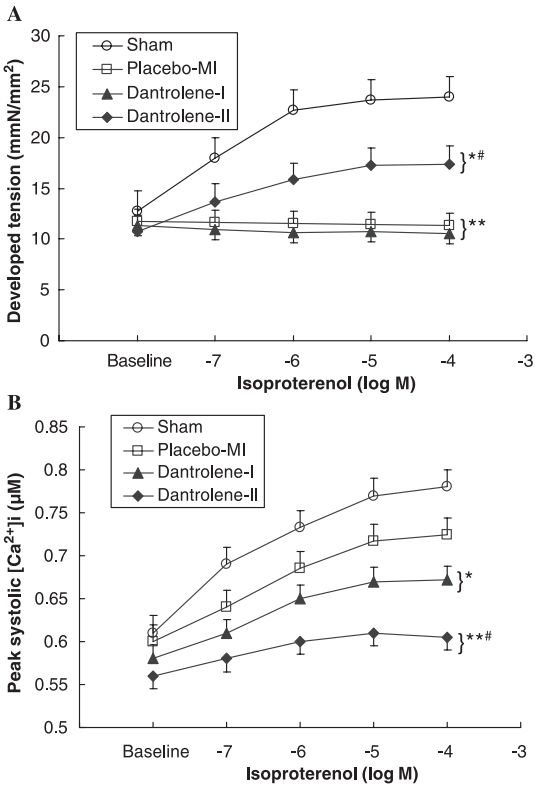

Fig. 1. Effects of dantrolene on (A) developed tension and (B) peak systolic intracellular Ca^{2+} in papillary muscles from postinfarction rats. $N=8$ in each group. $^{*}P<0.05$, $^{**}P<0.01$ vs. Sham at isoproterenol stimulation; $^{\#}P<0.01$ vs. Placebo-MI and Dantrolene-I at isoproterenol stimulation.

hypertrophy was attenuated with high dose of dantrolene treatment, i.e., in Dantrolene-II (Table 1). This finding suggested that a high dose of dantrolene attenuated the progress of ventricular hypertrophy after infarction. Additionally, high-dose treatment with dantrolene moderately enhanced mean arterial blood pressure. Heart rate showed no significant difference among the four experimental groups. Table 2 shows the baseline condition of mechanical performance and intracellular Ca^{2+} concentration in each group. Papillary muscles from placebo- and dantrolene-treated infarcted rats presented a similar reduction of developed tension and prolongation of time courses of contraction and relaxation at baseline compared to the sham group. Time to peak light signal and time from peak light to 50% decline in aequorin signal were prolonged in papillary muscles isolated from placebo- and low-dose dantrolene-treated infarcted rats. Treatment with a high dose of dantrolene in infarcted rats resulted in partial normalization of time from peak light to 50% decline in aequorin transient.

A graded increase in isoproterenol concentration resulted in significant enhancement of developed tension in papillary muscles isolated from sham-operated rats in parallel with an increase of intracellular Ca^{2+} transients (Figs. 1 and 2). However, this positive inotropic effect to isoproterenol stimulation was markedly blunted in placebo-treated infarcted rat papillary muscles despite an increase in peak amplitude of intracellular Ca^{2+} transients. The inotropic responses of muscle contractility and intracellular Ca^{2+} to isoproterenol stimulation were similar in the Dantrolene-I group with low-dose administration and the Placebo-MI group. High dose of dantrolene treatment in infarcted rats resulted in a significantly improved inotropic response to isoproterenol stimulation compared to placebo-treated and low-dose dantrolene-treated groups (Fig. 1), but no accompanying parallel increase in intracellular Ca^{2+} transient amplitude was observed. These results demonstrated that the high-dose treatment of dantrolene in infarcted rats partially restored the inotropic response to

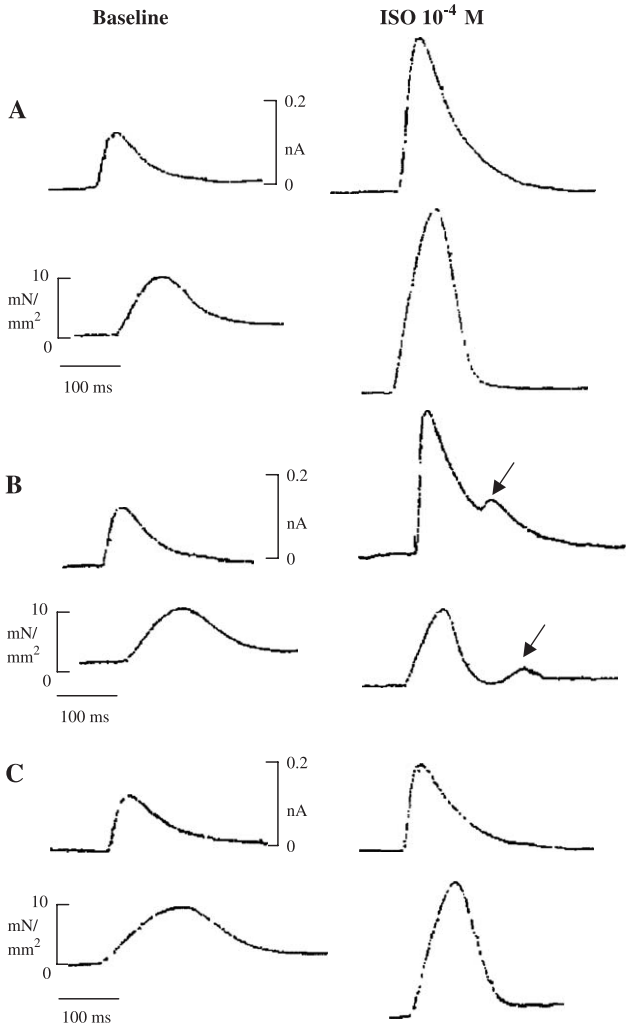

Fig. 2. Representative examples of aequorin light signal and isometric contraction from (A) a sham-operated rat papillary muscle, (B) a placebo-treated papillary muscle isolated from a rat 6 weeks after myocardial infarction during isoproterenol stimulation, and (C) a papillary muscle isolated from a rat 6 weeks after myocardial infarction treated with dantrolene (5 mg/kg, i.p.) during isoproterenol stimulation. Arrows point out the afterglimmer and aftercontraction at 10^{-4} M isoproterenol in panel B. ISO, at 10^{-4} M isoproterenol stimulation. Upper trace of each panel: aequorin light signal. Lower trace of each panel: isometric contraction.

isoproterenol stimulation, but this effect is not accompanied by an additional increase in intracellular Ca^{2+} availability.

The papillary muscles isolated from placebo-treated infarcted rats (three out of eight) exhibited afterglimmers and corresponding aftercontractions at high concentrations of isoproterenol (Fig. 2B). Chronic treatment with a high dose of dantrolene prevented the diastolic Ca^{2+} oscillations during isoproterenol stimulation (Fig. 2C). Diastolic intracellular Ca^{2+} concentration was remarkably increased in papillary muscles isolated from placebo- and low-dose dantrolene-treated infarcted rats (Table 2). Administration of high dose of dantrolene, i.e., in Dantrolene-II, significantly reduced abnormal increased diastolic Ca^{2+} in postinfarcted rat myocardium. Additionally, a high concentration of isoproterenol during the β-adrenoceptor stimulation in the placebo control group resulted in an increase in diastolic intracellular Ca^{2+} (0.35 ± 0.05 μM at baseline vs. 0.41 ± 0.06 μM at 10^{-4} M isoproterenol; $P < 0.05$). Increase of the diastolic intracellular Ca^{2+} at high concentration of isoproterenol was also found in the group of Dantrolene-I (0.34 ± 0.04 μM at baseline vs. 0.39 ± 0.03 μM at 10^{-4} M isoproterenol; $P < 0.05$). However, there is no significant change in diastolic intracellular Ca^{2+} in the groups of Sham (0.28 ± 0.03 μM at baseline vs. 0.28 ± 0.04 μM at 10^{-4} M isoproterenol) and Dantrolene-II (0.29 ± 0.02 μM at baseline vs. 0.30 ± 0.03 μM at 10^{-4} M isoproterenol).

Time courses of isometric twitches and relaxation exhibited shortening during isoproterenol stimulation in papillary muscles isolated from sham and postinfarcted rats. No significant changes in time courses of aequorin light signals were found during isoproterenol stimulation in all muscle preparations. The time courses of mechanical performance and intracellular Ca^{2+} transient were not significantly different in papillary muscles isolated from low or high dose of dantrolene administered and placebo-treated infarcted rats during β-adrenoceptor stimulation (data not shown).

The effects of myocardial infarction and dantrolene treatment on the protein levels of SERCA2 and phospholamban expression were measured in additional left ventricles (five for each group) from sham-operated rats, placebo-treated rats, low-dose dantrolene (2 mg/kg, i.p.)-treated infarcted rats, and high-dose dantrolene (5 mg/kg, i.p.)-treated rats. The protein levels of SERCA2 and phospholamban were analyzed as a ratio compared to cyclophilin. As shown in Fig. 3, there was a moderate reduction of protein levels of SERCA2 in placebo-treated and low-dose dantrolene-treated postinfarcted left ventricles compared to that from sham-operated rats. High dose of dantrolene treatment resulted in partially restored protein levels of SERCA2 in left ventricles from 6-week postinfarcted rats. In contrast, there was no significant difference in the protein levels of phospholamban in left ventricles from sham-operated rats, placebo-treated infarcted rats, and dantrolene-treated infarcted rats.

4. Discussion

Abnormal intracellular Ca^{2+} handling has been suggested as a major source of contractile dysfunction in failing myocardium (Morgan and Morgan, 1984; Morgan et al., 1990; Morgan, 1991; Gwathmey et al., 1987). In particular, elevation of diastolic intracellular Ca^{2+} may generate temporal and spatial inhomogeneities of intracellular Ca^{2+}, which, in turn, increase diastolic tone and reduce systolic force. This impairment has been attributed to alterations of the sarcoplasmic reticulum Ca^{2+} pump

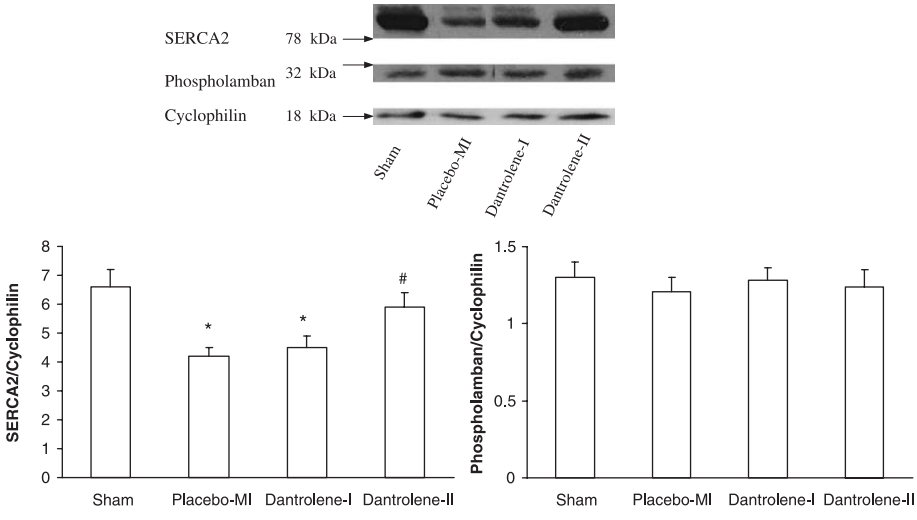

Fig. 3. Protein levels of SERCA2 and phospholamban in rat left ventricles from sham-operated and postinfarcted rats. Upper trace, Western blot; lower left, densitometric analysis of SERCA2/cyclophilin; lower right, densitometric analysis of phospholamban/cyclophilin. $N = 5$ in each group. $^{*}P < 0.05$ vs. Sham; $^{\#}P < 0.05$ vs. Placebo-MI.

and/or Ca^{2+} release channels (Hasenfuss et al., 1994; Schmidt et al., 1998; Min et al., 2001). Inadequate regulation of the gating mechanism may result in uncoordinated sarcoplasmic reticulum Ca^{2+} efflux, diastolic Ca^{2+} oscillations and an increase in diastolic intracellular Ca^{2+}. Previous studies demonstrated that spontaneous Ca^{2+} release from the sarcoplasmic reticulum during diastole is the primary cause of Ca^{2+} overload and subsequently induces contractile dysfunction (Allen et al., 1985). A variety of animal models of cardiac failure and human myocardium with heart dysfunction (Morgan et al., 1984; Gwathmey et al., 1987; Bing et al., 1991; Wikman-Coffel et al., 1991; Meissner et al., 1996) showed that the calcium transients recorded from failing myocardium had not only a prolonged time courses, which predominantly reflects Ca^{2+} handling by the sarcoplasmic reticulum, but also consisted of two distinct components (i.e., afterglimmers). Development of afterglimmers during isoproterenol stimulation in postinfarcted rat myocardium in the present study demonstrated the spontaneous Ca^{2+} oscillations during high concentrations of isoproterenol. Reduction in the protein levels of SERCA2 in placebo-treated infarcted rat myocardium suggested that sarcoplasmic reticulum function contributes to impaired intracellular Ca^{2+} handling.

The present study found that diastolic intracellular Ca^{2+} increased in postinfarcted rat myocardium. During β-adrenoceptor stimulation, the amplitude of peak systolic intracellular Ca^{2+} increased while systolic developed tension paradoxically did not in rat papillary muscles after infarction, which is consistent with our previous reports (Meissner et al., 1996; Min et al., 1999, 2000, 2001) and Allen et al. (1985). Afterglimmers and aftercontractions were observed in three out of eight muscle preparations in the present study, which is associated with the oscillatory release of Ca^{2+} from intracellular stores (Morgan and Morgan, 1984). Diastolic Ca^{2+} oscillations could result in inhomogeneities of diastolic sarcoplasmic reticulum Ca^{2+} handling, heterogeneous myofilament interaction and different diastolic sarcomere lengths, thereby reducing cardiac function. In the present study with rat papillary muscles after infarction, abnormal intracellular Ca^{2+} handling was augmented by β-adrenoceptor stimulation which unmasked potential signs of intracellular Ca^{2+} dysregulation, i.e., afterglimmers in aequorin light transients, and a subsequent blunted inotropic response to β-adrenoceptor stimulation. This result further supported the hypothesis that impaired intracellular Ca^{2+} homeostasis is, at least partially, responsible for reducing cardiac muscle force generation and attenuating the inotropic effect to isoproterenol stimulation in postinfarcted myocardium.

Dantrolene is known to inhibit Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulumin in skeletal muscle, which is an important mechanism as the cause of muscle rigidity in malignant hyperthermia (Ohta et al., 1990). This drug is also reported to be clinically effective for the treatment of malignant hyperthermia in humans (Kolb et al., 1982) and inhibits the Ca-induced Ca^{2+} release mechanism in skinned skeletal muscle of malignant hyperthermia-susceptible pigs (Ohta et al., 1989). Ca-induced Ca^{2+} release is not the physiological Ca^{2+} release mechanism in skeletal muscle, but it is important as the regulatory mechanism of excitation–contraction coupling in cardiac muscle (Fabiato, 1983), and secondarily to induce diastolic Ca^{2+} oscillations. The present study demonstrated that dantrolene was quite effective in reducing diastolic Ca^{2+} oscillations, thereby decreasing the magnitude of diastolic intracellular Ca^{2+}, and partially restoring the inotropic responsiveness to isoproterenol stimulation despite no accompanying parallel increase in peak systolic intracellular Ca^{2+} in rat papillary muscles after infarction. Additionally, dantrolene may enhance the myofilament Ca^{2+} sensitivity to a certain level, and then cause improvement of myocardial contractility. Further experiments are required to fully understand the mechanism of the beneficial effects of dantrolene on postinfarcted myocardium.

Our previous study (Meissner et al., 1996) in isolated rat cardiac sarcoplasmic reticulum with Ca^{2+} overload found that dantrolene increased the sarcoplasmic reticulum uptake, indicating that dantrolene can modulate the sarcoplasmic reticulum Ca^{2+} pumping activity. In the present study, administration of a high dose of dantrolene resulted in a decrease of the increased amplitude of diastolic intracellular Ca^{2+} and diastolic $[Ca^{2+}]_i$ oscillations in postinfarcted rat myocardium. In addition, dantrolene partially restored β-adrenoceptor responsiveness in rat myocardium after infarction. The benefits from dantrolene treatment are, at least partially, related to preservation of the sarcoplasmic reticulum function reflected by restoring the protein levels of SERCA2 in the present study. This phenomenon is consistent with a previous report by Ohkusa et al. (1998), suggesting that intracellular Ca^{2+} handling which is regulated by the sarcoplasmic reticulum might be the final signal in the development of hypertrophy and may play a crucial role in its progression. The present study demonstrates that chronic treatment with high dose of dantrolene reduced compensatory left ventricular hypertrophy compared to placebo-treated rats in the postinfarction model. This finding may reflect a beneficial effect on the myocardial remodeling process with treatment of dantrolene. However, it cannot fully explain the greater improvement of myocardial contractility and β-adrenoceptor responsiveness with high dose of dantrolene treatment.

Our data show for the first time that dantrolene affects the subcellular mechanisms of intracellular Ca^{2+} handling, reduces the increased diastolic intracellular Ca^{2+} availability in rat myocardium after myocardial infarction, and subsequently improves β-adrenoceptor responsiveness. These special beneficial effects of dantrolene appear to result, at least in part, from modification of the sarcoplasmic reticulum function. Thus, dantrolene should play a valuable therapeutic role for failing heart by altering Ca^{2+} homeostasis.

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