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A role of myofilament Ca^{2+} sensitivity in enhanced vascular reactivity in cardiomyopathic hamsters

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Abstract

We compared the contractile responses to vasoconstrictors in aortas from 20- to 22-week old cardiomyopathic hamsters, BIO 53.58 strain, and age-matched F1b strain controls. Aortas from cardiomyopathic hamsters exhibited greater contractions in response to phenylephrine, angiotensin II, and high K^+ than did the controls. Neither endothelium removal nor the presence of indomethacin and N^{ω}-nitro-L-arginine (L-NNA) affected the enhanced contractile responses to these vasoconstrictors, indicating no involvement of endogenous prostanoids and nitric oxide from the endothelium. The contractile response to phorbol-12,13-dibutyrate (PDB) was also more markedly increased in cardiomyopathic aortas regardless of whether extracellular Ca^{2+} was present. The contractile response of cardiomyopathic aorta to phenylephrine was more sensitive to the inhibitory actions of the protein kinase C inhibitors staurosporine and calphostin C than was that of control aorta. These results suggest that activation of protein kinase C is partly involved in the enhanced phenylephrine response of cardiomyopathic aorta. None of nifedipine, ryanodine, and cyclopiazonic acid modified the maximum contractions induced by phenylephrine in either cardiomyopathic aortas or controls. The Ca^{2+} sensitivity of tension was significantly increased in β-escin-skinned smooth muscle of mesenteric artery from cardiomyopathic hamsters compared to that of controls. PDB induced Ca^{2+} sensitization, but significantly only in cardiomyopathic hamsters. We propose that the enhanced vascular reactivity in cardiomyopathic hamsters may primarily result from increased Ca^{2+} sensitivity of contractile proteins. In addition, protein kinase C-mediated Ca^{2+} sensitization may further contribute to the enhanced vascular response to agonists.

Keywords: Congestive heart failure; Cardiomyopathic hamster; Vascular contractility; Protein kinase C; Myofilament; Ca^{2+} sensitivity; Smooth muscle, vascular

1. Introduction

The chronically reduced cardiac output caused by the depressed cardiac performance observed in congestive heart failure promotes activation of the sympathetic nervous system and the renin–angiotensin system, as well as the release of arginine vasopressin (Francis, 1985). This autonomic or hormonal adjustment may initially act to compensate the depressed cardiac performance and to maintain perfusion in vital organs, but ultimately aggravates the failure of left ventricular function (Francis, 1985). In addition to increased amounts of vasoconstrictive hormones, alterations in vascular reactivity to these hormones may contribute to an increase in systemic vascular resistance in congestive heart failure. Forster and her coworkers (Forster et al., 1989; Forster and Armstrong, 1990; Forster and Campbell, 1993) have shown that the contractile responses of dorsal pedal artery and saphenous vein to α_1-adrenoceptor agonists are enhanced in pacing-induced heart failure in dogs. Furthermore, it has been demonstrated that the coronary vasoconstrictor response to intracoronary infusion of the endothelin-B receptor agonist, sarafotoxin, is significantly enhanced in dogs with congestive heart failure induced by thoracic inferior vena cava constriction (Cannan et al., 1996). In contrast, in vivo and in vitro vascular responses to α_2-adrenoceptor agonists have been reported to be decreased in rats with congestive heart failure produced by coronary artery ligation (Feng et al., 1996). Porsa

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et al. (1994) have found that the contractile responses of aortic rings to high K^+, phenylephrine, and angiotensin II are unchanged or decreased in rabbits subjected to tachycardia heart failure. Thus alterations in vascular reactivity to vasoconstrictors are variable, depending on the animal model used.

The cardiomyopathic Syrian hamster is known to develop a genetically determined cardiomyopathy, with development of progressive and ultimately fatal congestive heart failure (Gertz, 1972). It has been used for many years as an animal model of human idiopathic dilated cardiomyopathy and the resulting heart failure. Although this model has been much used for studies addressing myocardial abnormalities in heart failure, limited information is available on how vascular reactivity is altered in this animal model. A few studies have revealed that the blood vessels isolated from cardiomyopathic hamsters are hyperresponsive to various vasoconstrictors compared to controls (Hunter and Elbrink, 1983; Dumont et al., 1996). Here we confirm and extend these previous reports by demonstrating more clearly that aortas from cardiomyopathic hamsters exhibit enhanced contractility in response to phenylephrine, angiotensin II, and high K^+. Additional experiments were carried out to explore possible mechanisms underlying the enhanced vascular reactivity in cardiomyopathic hamsters.

2. Methods

2.1. Study design

Animals were obtained from Bio Breeders (Fitchburg, MA) and maintained under constant temperature (23°C) and lighting conditions (lights on from 0600 to 1800 h) with free access to food and water. Male cardiomyopathic Syrian hamsters of the BIO 53.58 strain were used at 20–22 weeks of age, a time when they showed ventricular dysfunction with a percentage fractional shortening of ~ 20% on echocardiography (Urasawa et al., 1996). Hamsters of the F1b strain, matched for age, served as controls.

2.2. Isometric contraction experiments

This work was approved by the Hokkaido University School of Medicine Animal Care and Use Committee. The animals were lightly anaesthetized with diethyl ether. Thoracic aortas were carefully excised from open-chest animals and placed in an oxygenated bathing medium. Each aorta was cleaned of loosely adhering fat and connective tissue and cut into rings of 3 mm length. In some experiments, the superior mesenteric artery was isolated and prepared as described above. Each ring was suspended by a pair of stainless-steel hooks under a resting tension of 1.5 g for aorta and 0.75 g for mesenteric artery in a water-jacketed bath filled with 25 ml of normal physiological salt solution (PSS). The resting tension was determined to be optimal for both control and cardiomyopathic arteries. The composition of the normal PSS was as follows (mM): NaCl 118.2, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 10.0. High-K^+ PSS was made by replacing NaCl with equimolar KCl. The solution in the bath was gassed with 95% O_2 and 5% CO_2, and its temperature was maintained at 37°C. Force generation was monitored using an isometric transducer (45196, Sanei-Sokki, Tokyo, Japan) and a carrier amplifier (1236, Sanei-Sokki). The output of the force transducer was recorded using a pen recorder (ERP-241A, TOA Electronics, Tokyo, Japan), through a polygraph recorder (142-8, Sanei-Sokki).

Following an equilibration period of at least 60 min, the rings were exposed several times to 40 mM K^+ until reproducible contractile responses were obtained. This procedure ensured that the preparation had been stabilized sufficiently to establish concentration–response curves for various vasoconstrictors. Experiments were usually performed in the presence of 10 μM indomethacin and 100 μM N^{ω}-nitro-L-arginine (L-NNA) to inhibit formation of endogenous prostanoids and nitric oxide unless otherwise stated. Concentration–response curves for phenylephrine- and angiotensin II-induced contractions were obtained by a cumulative increase in the concentration of the agents in the tissue chamber. For establishment of the curve for K^+-induced contractions, KCl was added cumulatively and no osmotic adjustment was made. The tissue was exposed to each concentration of the agents until the contractile response reached a plateau, which usually occurred within 10 min. On completion of the concentration–response curve for phenylephrine, the rings were thoroughly washed with normal PSS and then incubated with 20 nM staurosporine, 1 μM calphostin C, 1 μM nifedipine, 10 μM ryanodine or 10 μM cyclopiazonic acid, for 30 min. The concentration–response curve for phenylephrine was repeated in the presence of each of the compounds. The contractile response to 1 μM phorbol-12,13-dibutyrate (PDB) was obtained in the normal PSS or Ca^{2+}-free PSS. Ca^{2+}-free PSS was made by omitting $CaCl_2$ and adding 1 mM EGTA.

After the completion of each procedure, the rings were carefully blotted dry and weighed. Contractile responses were expressed as milligrams of tension developed per milligram tissue wet weight to account for the differences in the cross-sectional area of the ring preparation.

2.3. Experiment on skinned smooth muscle

Skinned preparations were made by treating vascular smooth muscle with β-escin. The method used for skinning and the composition of the solutions have been described elsewhere (Tomita et al., 1997). Since it was found quite difficult to permeabilize successfully hamster aortas in spite of repeated challenges, we used mesenteric arteries instead of aortas isolated from control and car-

diomyopathic hamsters for this experiment. A small bundle of muscle fibers (1 mm length and 0.5 mm width) was prepared, its ends were tied with monofilament silk to the fine tips of two tungsten needles, one of which was connected to a lever of the force transducer (TB 651-T, Nihon Kohden, Tokyo, Japan) movable perpendicularly and the other was fixed on a supporting bar sliding horizontally and perpendicularly. The whole of the preparation was placed in a well with 0.75 ml of the normal PSS at room temperature (22–25°C). The bundles were stretched to about 1.2 times their resting length. Then, the preparations were treated with 50 μM β-escin for 30 min in a relaxing solution containing 87 mM potassium methanesulphonate, 20 mM PIPES, 5.1 mM Mg(methanesulphonate)$_2$, 4.2 mM ATP, 10 mM phosphocreatine, 0.5 mg/ml creatine phosphokinase and 10 mM EGTA (pH 7.0). After the skinning procedure, the preparations were washed to remove β-escin by replacement of the normal relaxing solution in the well.

To determine the contractile response of the skinned muscle to Ca^{2+}, various concentrations of Ca^{2+} were applied by adding appropriate amounts of Ca(methanesulphonate)$_2$ to the relaxing solution. The pH of the solution was adjusted to 7.1 with KOH and the ionic strength was kept constant at 0.2 M by changing the amount of potassium methanesulphonate added. To avoid spurious effects due to Ca^{2+} release from intracellular storage sites in the skinned muscle, 1 μM ionomycin was applied. To prevent deterioration of the Ca^{2+}-induced contraction, 0.1 μM calmodulin was applied throughout the experiments. The effect of a higher concentration of calmodulin (1 μM) was confirmed to be similar to that of 0.1 μM calmodulin. When the Ca^{2+}–tension relationship was to be determined, various concentrations of Ca^{2+} were applied cumulatively from low to high concentrations. The amplitude of contraction induced by each of the concentrations of Ca^{2+} tested was normalized by taking the maximal tension developed in the same preparation as 1.0. The EC_{50} value, i.e., the Ca^{2+} concentration giving half-maximal activation, was determined from log–probit plots of the individual response vs. concentrations and was expressed as negative logarithm (pCa_{50}). The data for the normalized pCa–tension relationship were applied to the Hill equation and the Hill coefficient was computed as:

$$F/F_{max} = [Ca^{2+}]^{n_H}/\left([EC_{50}]^{n_H} + [Ca^{2+}]^{n_H}\right),$$

where F_{max} is the maximally activated tension, F is the tension developed at the actual Ca^{2+} concentration, and n_H is the Hill coefficient that is an index of cooperativity.

2.4. Drugs

The compounds used were as follows: L-phenylephrine hydrochloride, angiotensin II, indomethacin, L-NNA, calphostin C, nifedipine, ryanodine, cyclopiazonic acid, PDB, β-escin and ionomycin (Sigma, St. Louis, MO, USA), staurosporine (Kyowa Hakko, Tokyo, Japan) and calmodulin (Wako, Osaka, Japan). Indomethacin, calphostin C, staurosporine, PDB, ryanodine and cyclopiazonic acid were prepared in dimethyl sulfoxide. Nifedipine and L-NNA were dissolved in absolute ethanol and 0.3 M HCl, respectively. Further dilutions were made with PSS. The final concentration of the solvents in PSS was always less than 0.1%. The experiments with nifedipine were performed in the dark, and solution bottles and tubing were covered with aluminum foil as further precaution against degradation.

2.5. Statistical analysis

All values are expressed as means ± S.E.M. Student's t-test was used to make comparisons between control and cardiomyopathic groups. The analyses were carried out using the software Stat View (Abacus Concepts). A P-value < 0.05 was considered statistically significant.

3. Results

3.1. General features

At the age of 20–22 weeks the body weights of cardiomyopathic hamsters (108 ± 1 g, $n = 55$) were significantly lower than those of control animals (143 ± 2 g, $n = 56$; $P < 0.01$). Furthermore, the heart weight to body weight ratios were significantly lower in cardiomyopathic hamsters than in control animals (2.55 ± 0.04 vs. 2.72 ± 0.02 mg/g, $n = 27$ and 28; $P < 0.01$). Additionally, the wet weight of aortic rings from cardiomyopathic hamsters (1.43 ± 0.05 mg, $n = 55$) was significantly less than that from control animals (1.72 ± 0.06 mg, $n = 52$; $P < 0.001$).

3.2. Contractile responses to phenylephrine, angiotensin II and high K^+

Typical tracings of the contractile responses of aortic and mesenteric arterial rings from control and cardiomyopathic hamsters to 1 μM phenylephrine are depicted in Fig. 1A. The contraction produced by phenylephrine was typically greater in aorta and mesenteric artery from cardiomyopathic hamsters compared to that of control arteries. The increase in tension in response to 1 μM phenylephrine in cardiomyopathic mesenteric arteries was 423 ± 72 mg/mg wet weight ($n = 4$) which was significantly greater than the corresponding value of 224 ± 26 mg/mg wet weight ($n = 4$; $P < 0.05$). Fig. 1B shows the concentration–response curves for phenylephrine-induced contractions of aortic rings from control and cardiomyopathic hamsters. The maximum response to phenylephrine in cardiomyopathic aortas was about three times greater than that in controls. In contrast, the sensitivity of aortas to

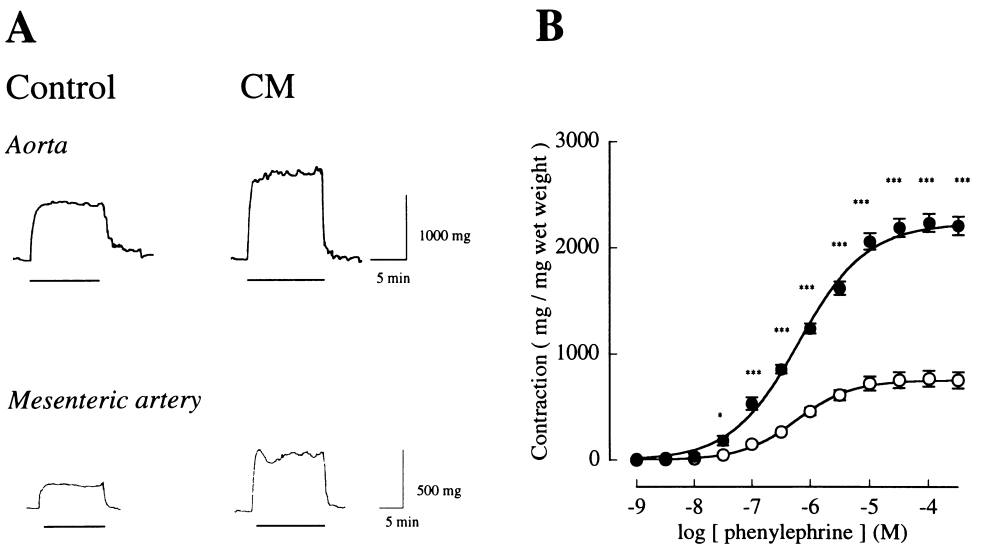

Fig. 1. Phenylephrine-induced contractions in mesenteric arteries and aortas from control and cardiomyopathic hamsters. (A) Representative tracings showing the contractile response of 1 μM phenylephrine in aortas (upper panels) and mesenteric arteries (lower panels) from control and cardiomyopathic (CM) hamsters. (B) Concentration–response curves for phenylephrine-induced contractions in aortas from control (○) and cardiomyopathic (●) hamsters. Points are means ± S.E. of six experiments. $* P < 0.05$, $* * * P < 0.001$ compared with corresponding control values.

phenylephrine (as reflected by pD_2 value, i.e., $-\log EC_{50}$) was essentially the same in the two groups (cardiomyopathic: 6.22 ± 0.06, control: 6.27 ± 0.08). Even after mechanical removal of the endothelium, a marked difference was similarly noted in the responsiveness of aortas from cardiomyopathic and control hamsters (Fig. 2A). Furthermore, when 10 μM indomethacin and 100 μM L-NNA were added as pretreatment, cardiomyopathic aortas showed greater contractions in response to phenylephrine than did the controls (Fig. 2B).

Angiotensin II and high K^+ also induced greater contractions in aortas from cardiomyopathic hamsters than in control preparations. The maximum response to angiotensin II was almost doubled in cardiomyopathic aortas compared to controls (1992 ± 197 vs. 1083 ± 117 mg/mg wet weight, $n = 4$ for each group; $P < 0.01$). The high K^+-induced maximum increase in tension in cardiomyopathic aortas was 2044 ± 99 mg/mg wet weight ($n = 6$) which was significantly greater than the corresponding value of 1137 ± 96 mg/mg wet weight ($n = 6$; $P < 0.001$) obtained in control preparations.

3.3. Contractile response to PDB and effects of staurosporine and calphostin C

Fig. 3A illustrates typical contractions of aortas from control and cardiomyopathic hamsters in response to 1 μM PDB, a direct activator of protein kinase C (Castagna et

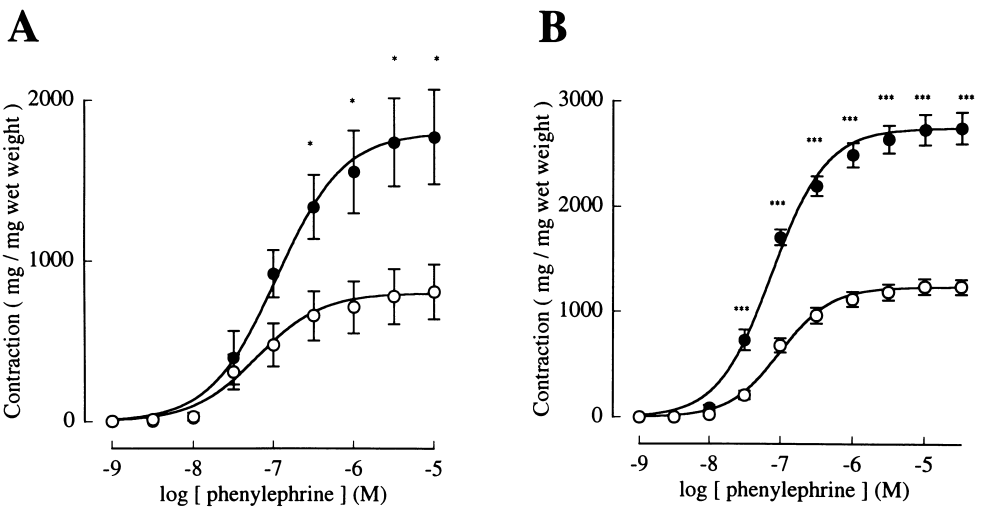

Fig. 2. Concentration–response curves for phenylephrine-induced contractions in aortas from control (○) and cardiomyopathic (●) hamsters after endothelium removal (A) and in the presence of 10 μM indomethacin and 100 μM L-NNA (B). Points are means ± S.E. of four to six experiments. $* P < 0.05$, $* * * P < 0.001$ compared with corresponding control values.

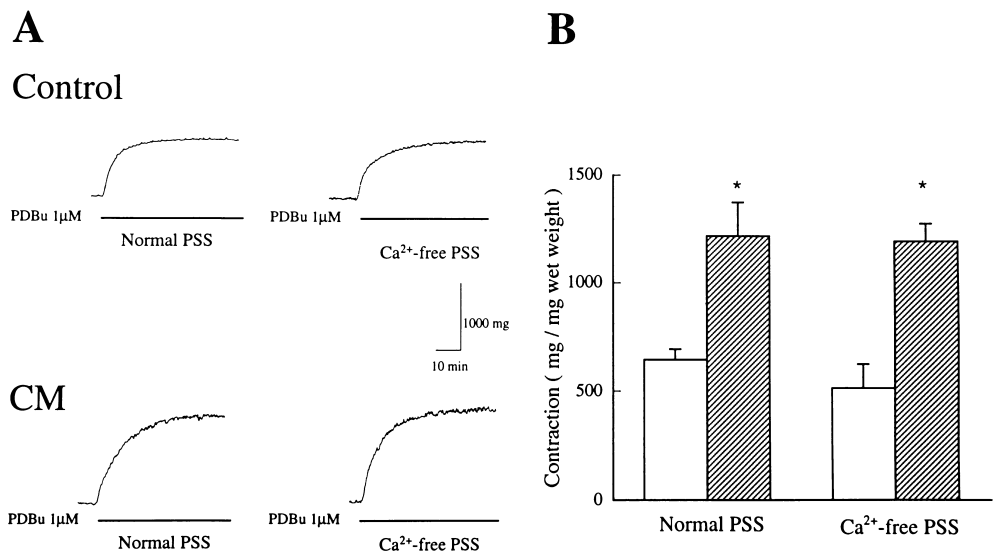

Fig. 3. Contractile responses to PDB in aortas from control and cardiomyopathic hamsters. All experiments were performed in the presence of 10 μM indomethacin and 100 μM L-NNA. (A) Typical tracings of contractions induced by 1 μM PDB under normal PSS and Ca^{2+}-free PSS in control (top) and cardiomyopathic (CM) (bottom) aortas. (B) Bar graph summarizing the data obtained as in panel A, and showing, as the mean ± S.E. ($n = 3–4$), the responses of control aortas (open bars) and those of cardiomyopathic aortas (hatched bars). * $P < 0.05$ compared with control.

al., 1982). In both control and cardiomyopathic aortas, PDB caused relatively slowly developing contractions independent of extracellular Ca^{2+}. The responses induced by PDB in Ca^{2+}-free PSS were similar to those in normal PSS. The PDB-induced contractions were apparently greater in cardiomyopathic aortas than those in the controls, regardless of whether extracellular Ca^{2+} was present or not. The results of these experiments are summarized in Fig. 3B.

The finding that the contractile response to direct activation of protein kinase C was enhanced in cardiomyopathic aortas prompted us to test the effects of staurosporine and calphostin C, drugs with relative selectivity to inhibit protein kinase C (Tamaoki et al., 1986; Kobayashi et al., 1989), on phenylephrine-induced contractions in control and cardiomyopathic aortas. The concentrations of staurosporine (20 nM) and calphostin C (1 μM) that we used in this set of experiments were found to be enough to produce marked inhibition of contractions induced by 1 μM PDB (data not shown) in both control and cardiomyopathic aortas, indicating effective antagonism of protein kinase C-mediated responses of aortas. As shown in

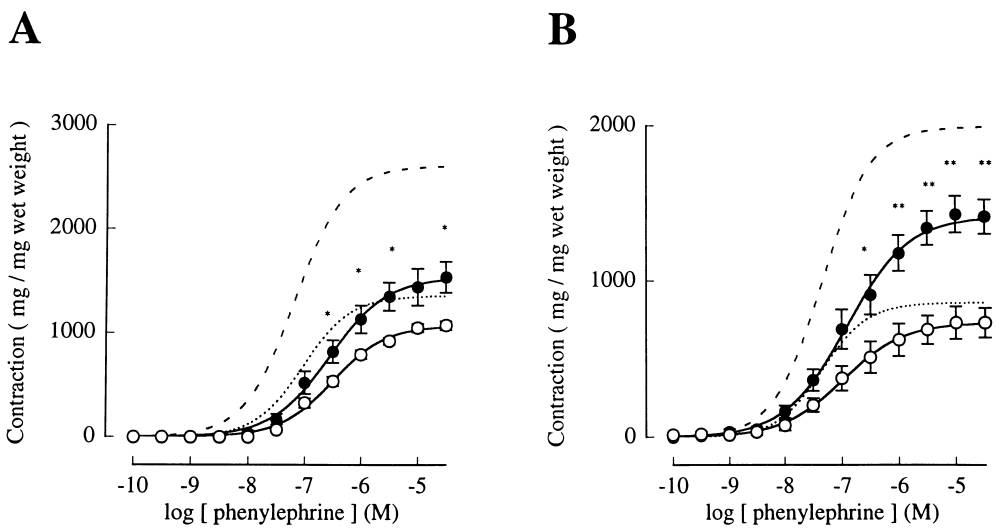

Fig. 4. Effects of 20 nM staurosporine (A) and 1 μM calphostin C (B) on concentration–response curves for phenylephrine-induced contractions in aortas from control (○) and cardiomyopathic (●) hamsters. Dotted and broken curves indicate the phenylephrine response before treatment with staurosporine or calphostin C in control and cardiomyopathic aortas, respectively. All experiments were performed in the presence of 10 μM indomethacin and 100 μM L-NNA. Points are means ± S.E. of five to six experiments. * $P < 0.05$, * * $P < 0.01$ compared with corresponding control values.

Table 1
Effects of nifedipine, ryanodine and cyclopiazonic acid on the contractile response to phenylephrine in control and cardiomyopathic aortas

Treatment	Maximum response (mg/mg wet weight)	
	Control	Cardiomyopathic
Nifidipine		
Before	1278 ± 79	2611 ± 122[a]
After	1360 ± 161	2527 ± 108[a]
Ryanodine		
Before	1248 ± 71	2766 ± 184[a]
After	1275 ± 69	2529 ± 156[a]
Cyclopiazonic acid		
Before	1239 ± 108	2734 ± 149[a]
After	1103 ± 44	2549 ± 166[a]

Each value represents means ± S.E. of five to six experiments.
All data are derived from cumulative concentration–response curves before and after 1 μM nifedipine, 10 μM ryanodine or 10 μM cyclopiazonic acid.
There was no significant difference between the maximum responses to phenylephrine before and after each treatment.
[a] $P < 0.001$ compared with the corresponding values obtained in control aortas.

Fig. 4A, pretreatment with staurosporine caused a rightward and downward shift of the concentration–response curve for phenylephrine in both control and cardiomyopathic aortas. However, the reduction in phenylephrine-induced contractions was more marked in cardiomyopathic aortas. Thus staurosporine reduced the maximum response to phenylephrine by 21% in control and 41% in cardiomyopathic aortas. Pretreatment with calphostin C also shifted the curve to the right in aortas from both groups of animals, but the significant reduction in the maximum response was observed only in cardiomyopathic aortas (Fig. 4B). In the presence of calphostin C, the maximum response to phenylephrine was decreased by 9% in control and 31% in cardiomyopathic aortas. Nevertheless, even in the presence of these inhibitors, the contractile response of cardiomyopathic aortas to phenylephrine was still significantly greater than that of controls.

3.4. Influences of nifedipine, ryanodine and cyclopiazonic acid

To determine if the increased vascular responsiveness in cardiomyopathic hamsters was the result of an increased transmembrane Ca^{2+} influx or of an increased Ca^{2+} release from intracellular stores, we examined the effects of the chemical agents which modify intracellular Ca^{2+} dynamics on the contractile responses to phenylephrine in control and cardiomyopathic aortas.

Nifedipine, a dihydropyridine Ca^{2+} channel antagonist, at a concentration of 1 μM, nearly completely abolished the high K^+-induced contractions in aortas from both groups of animals. At this concentration, however, nifedipine marginally affected phenylephrine-induced contractions (Table 1). In both control and cardiomyopathic aortas, the maximum responses to phenylephrine in the presence of nifedipine were the same as those obtained in its absence.

Ryanodine depletes intracellular Ca^{2+} stores by opening Ca^{2+} release channels and making the endoplasmic reticulum leaky to Ca^{2+} (Hwang and Van Breemen, 1990). In neither control nor cardiomyopathic aortas did pretreat-

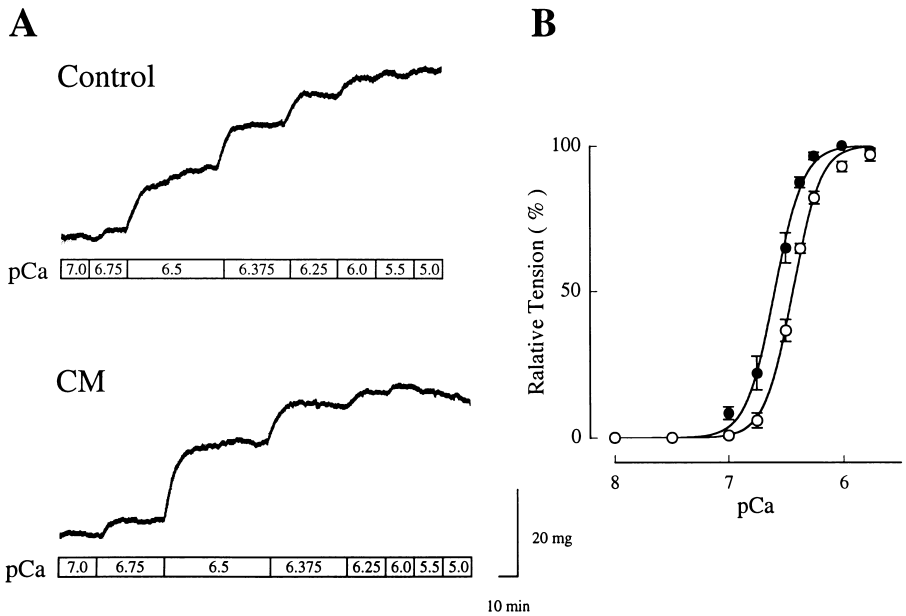

Fig. 5. The *p*Ca–tension relationships in β-escin-skinned smooth muscle of mesenteric arteries from control and cardiomyopathic hamsters. (A) Represents typical tracings of concentration-dependent Ca^{2+}-induced contractions in control (top) and cardiomyopathic (CM) (bottom) preparations. (B) Represents overall results of four different experiments. Control, ○; Cardiomyopathic, ●.

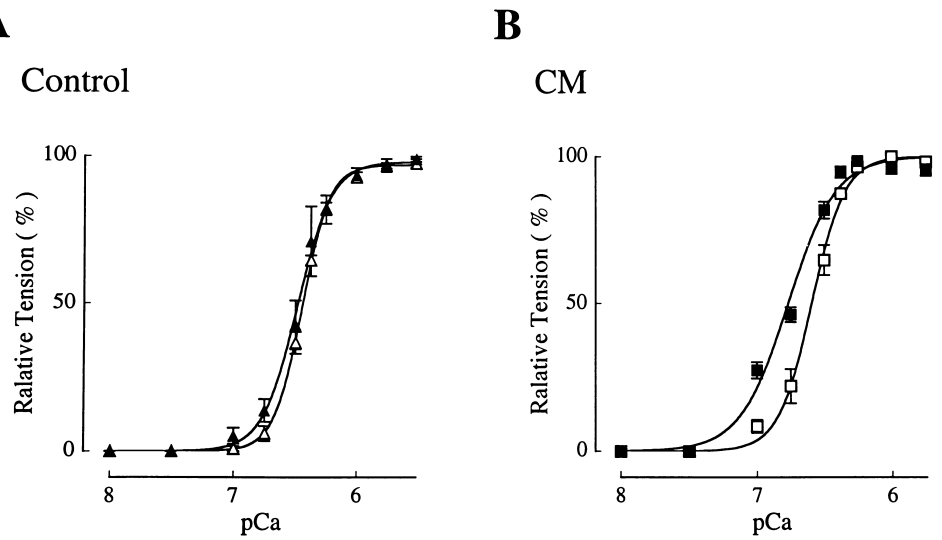

Fig. 6. The *p*Ca–tension relationships in the absence (△, □) and presence of 1 μM PDB (▲, ■) in β-escin-skinned smooth muscle of mesenteric arteries from control (A) and cardiomyopathic (B) hamsters. Points are means ± S.E. of four different experiments.

ment with 10 μM ryanodine result in a significant change in the maximum response or sensitivity to phenylephrine (Table 1).

Cyclopiazonic acid reduces intracellular Ca^{2+} stores by inhibiting the Ca^{2+}-pump adenosinetriphosphatase (ATPase) of the endoplasmic reticulum (Georger et al., 1988). Pretreatment with 10 μM cyclopiazonic acid significantly increased the sensitivity to phenylephrine in aortas from both groups; the pD_2 value was increased from 6.99 ± 0.04 to 7.66 ± 0.20 in control and from 7.12 ± 0.05 to 7.50 ± 0.04 in cardiomyopathic aortas after treatment with cyclopiazonic acid ($n = 6$ for each). However, the maximum responses to phenylephrine were not affected by this treatment (Table 1).

Thus, cardiomyopathic aortas developed greater (~ 100%) contractile tension in response to phenylephrine than did the controls in spite of the presence of nifedipine, ryanodine, and cyclopiazonic acid.

3.5. Ca^{2+} sensitization of vascular smooth muscle

Fig. 5A shows representative tracings of Ca^{2+}-induced contractions in β-escin-skinned smooth muscle of mesenteric arteries from control and cardiomyopathic hamsters. Ca^{2+} induced contraction in preparations from both groups of animals in a concentration-dependent manner. However, the muscle preparation from cardiomyopathic hamsters developed a greater tension at given Ca^{2+} concentrations compared to control preparations. The averaged *p*Ca–tension relationships are shown in Fig. 5B. The maximal tension was unchanged, with muscle preparations from control and cardiomyopathic hamsters developing 28 ± 5 ($n = 4$) and 39 ± 1 mg ($n = 4$) of tension respectively. Tensions at submaximal concentrations of Ca^{2+} were significantly higher in cardiomyopathic preparations than in controls. The increase in Ca^{2+} sensitivity of tension was consistently observed in the range of *p*Ca values from 7 to 6. The pCa_{50} value was 6.43 ± 0.02 for control and 6.61 ± 0.04 for cardiomyopathic preparations ($P < 0.01$). The slope of the *p*Ca–tension curve was not significantly different between the two preparations. The Hill coefficients were 3.54 ± 0.34 for control and 3.44 ± 0.27 for cardiomyopathic preparations.

PDB at a concentration of 1 μM slightly shifted the *p*Ca–tension curve to the left in vascular smooth muscle preparations from control hamsters (Fig. 6A). However, this shift induced by PDB was not statistically significant, as indicated by the pCa_{50} value of 6.47 ± 0.06 ($n = 4$). In contrast, PDB caused a significant leftward shift of the *p*Ca–tension curve in cardiomyopathic preparations, with no change in the maximum amplitude of contraction obtained at *p*Ca 5.75 (Fig. 6B). The pCa_{50} value obtained in the presence of PDB (6.77 ± 0.02, $n = 4$) was significantly higher than that in its absence ($P < 0.01$). The Hill coefficient was significantly decreased by PDB (2.33 ± 0.17; $P < 0.01$).

4. Discussion

The present study demonstrated that the contractile responses to phenylephrine, angiotensin II, and high K^+ were markedly enhanced in aortas from cardiomyopathic hamsters of the BIO 53.58 strain compared to those of age-matched controls (F1b). These results are in good agreement with previous findings that aortic strips from cardiomyopathic hamsters of the BIO 14.6 strain exhibit increased contractility in response to norepinephrine, phenylephrine, isoproterenol, histamine, and 5-hydroxytryptamine (Hunter and Elbrink, 1983). Our observations are also consistent with reports showing that cardiomyo-

pathic hamster arterioles respond excessively to vasoconstrictors such as norepinephrine and arginine vasopressin (Factor et al., 1982; Conway et al., 1987, 1994). However, the mechanisms underlying the increased contractility in response to different vasoconstrictors in cardiomyopathic hamster blood vessels are poorly understood. The current study addressed the question of what plays a causal role in the enhanced vascular reactivity in this animal model.

The endothelium has an important role in the modulation of vascular tone by releasing nitric oxide and prostacyclin in response to a variety of vasodilators (Furchgott and Vanhoutte, 1989). The endothelium-dependent vasorelaxant response to acetylcholine was shown to be impaired in the microcirculation of cardiomyopathic hamsters, as seen in cheek pouch arterioles (Mayhan and Rubinstein, 1992). However, it is unlikely that an altered endothelial function can participate as a contributory factor in the enhanced contractile reactivity in cardiomyopathic hamster aortas. Even after endothelium removal or incubation with the nitric oxide synthase inhibitor, L-NNA, and the cyclooxygenase inhibitor, indomethacin, the enhanced contractile responses of cardiomyopathic aortas to different vasoconstrictors were still evident. A recent study has shown that acetylcholine induces similar relaxations in phenylephrine-precontracted aortic rings from control and cardiomyopathic hamsters (Dumont et al., 1996). Thus it appears that the endothelium is intact and fully functional, at least in large conduit arteries.

High K^+ causes membrane depolarization and thereby leads to opening of L-type Ca^{2+} channels, allowing the influx of extracellular Ca^{2+} into the cell. According to this, the finding that high-K^+-induced contractions were greater in cardiomyopathic aortas compared to those from controls might suggest that the enhanced responses of cardiomyopathic aortas to vasoconstrictors resulted from an increased Ca^{2+} influx through Ca^{2+} channels. However, this does not appear to have been the case. Pretreatment with nifedipine produced no change in the magnitude of the contractile response to phenylephrine in aortas from control and cardiomyopathic aortas, indicating that activation of Ca^{2+} channels is not involved in production of the phenylephrine response of hamster aortas. Furthermore, the magnitude of the contractile response of cardiomyopathic aortas to phenylephrine in the presence of nifedipine was the same as that obtained in its absence. This implies that the enhanced responsiveness of cardiomyopathic aortas to phenylephrine is independent of Ca^{2+} entry through Ca^{2+} channels. We also found that PDB induced a greater contraction in cardiomyopathic aortas regardless of whether extracellular Ca^{2+} was present, which supports the view that the enhanced vascular contractile reactivity in cardiomyopathic aortas is not associated with activation of Ca^{2+} channels. It has been reported that the amount of [^{3}H]PN200-110 (isopropyl-4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-3-pyridine carboxylate) binding is not different in aortic smooth muscle cells from control and in those from cardiomyopathic hamsters (Tawada-Iwata et al., 1992).

Alterations in intracellular Ca^{2+} handling have been documented in cardiac and skeletal muscles from cardiomyopathic hamsters (Sulakhe and Dhalla, 1971; Jasmin and Solymoss, 1975). One may argue that changes in Ca^{2+} sequestration of the endoplasmic reticulum caused the enhancement of vascular reactivity observed in cardiomyopathic hamsters. This possibility was examined using ryanodine and cyclopiazonic acid. Neither of the agents affected the maximum response to phenylephrine in control and cardiomyopathic aortas, although cyclopiazonic acid significantly increased the sensitivity to phenylephrine in both aortas. Thus, the enhanced contractile response of cardiomyopathic aortas to phenylephrine was not modified by these agents. The results indicated that altered functions of the endoplasmic reticulum, if any, play a minor role in the increased vascular reactivity in cardiomyopathic hamsters.

The major finding of the present study was that cardiomyopathic hamsters exhibit an increase in Ca^{2+}-induced contractions with no change in the slope of the *p*Ca–tension relationship or maximum tension generated in β-escin-skinned smooth muscle of mesenteric artery. The different Ca^{2+} sensitivity observed in control and in cardiomyopathic skinned muscles could reflect a difference in the degree of contraction at a given cytosolic Ca^{2+} level. Thus, vascular smooth muscle of cardiomyopathic hamsters may develop a larger contraction for a given increase in cytosolic Ca^{2+} concentration in response to vasoconstrictors than do those of controls. Interestingly, the Ca^{2+} transients induced by norepinephrine and endothelin-1 in aortic smooth muscle cells from cardiomyopathic hamsters have been shown to be reduced rather than in control cells (Tawada-Iwata et al., 1992). The apparent discrepancy between this unexpected finding of the reduced Ca^{2+} transients and the enhanced contractions described by us and other investigators could be explained by the increased Ca^{2+} sensitivity of the contractile apparatus in cardiomyopathic hamsters. Several studies have addressed alterations in the Ca^{2+} sensitivity of cardiac muscles from cardiomyopathic hamsters. Heyder et al. (1995) have shown that skinned fibers from ventricles of cardiomyopathic hamsters are more Ca^{2+}-sensitive than are fibers from age-matched controls. These authors have also demonstrated that the Ca^{2+} sensitivity became similar in control and cardiomyopathic skinned cardiac fibers after troponin I extraction and reconstitution with bovine cardiac whole troponin, indicating that the differences in Ca^{2+} sensitivity may result from alterations in regulatory proteins. Indeed, Malhotra (1990) has suggested that troponins are altered in cardiomyopathic hamster hearts. Therefore, it is possible that altered regulatory proteins seen in cardiac muscles may also lead to the increased Ca^{2+} sensitivity of the vascular tension in cardiomyopathic hamsters. Before such a conclusion can be arrived

at, however, it must be determined whether the expression of troponin I and other regulatory proteins such as myosin light chains is altered in vascular smooth muscles of cardiomyopathic hamsters.

Activation of protein kinase C enhances the Ca^{2+} sensitivity of myofilaments in skinned smooth muscle preparations (Chatterjee and Tejada, 1986; Fujiwara et al., 1988; Nishimura and Van Breemen, 1989; Itoh et al., 1994). The present study showed that PDB definitely potentiated Ca^{2+} sensitivity more in cardiomyopathic vascular preparations than in the controls. Thus protein kinase C activation plays a certain role in regulating the Ca^{2+} sensitivity of vascular tension in cardiomyopathic hamsters. This could explain the finding that PDB produced greater contractions in cardiomyopathic aortas independently of extracellular Ca^{2+}. It is known that receptor agonists, including phenylephrine and angiotension II, stimulate the hydrolysis of phosphatidylinositol 4,5-bisphosphate through activation of phospholipase C, yielding diacylglycerol as well as inositol 1,4,5-trisphosphate, and diacylglycerol activates protein kinase C (Nishizuka, 1992). In the present study staurosporine inhibited the contractile response of cardiomyopathic aortas to phenylephrine more markedly than that of controls, whereas calphostin C significantly decreased the maximum response to phenylephrine only in cardiomyopathic aortas. This slight difference between the actions of the two inhibitors may be due to staurosporine actions unrelated to its inhibition of protein kinase C (Kageyama et al., 1991). These findings indicate that the contribution of activation of protein kinase C to the contractile response to phenylephrine is much greater in cardiomyopathic aortas than in the controls. However, it should be noted that the inhibition of the enhanced contractile response of cardiomyopathic aortas to phenylephrine by the protein kinase C inhibitors was incomplete. Therefore, it seems likely that part of the enhanced contractile response of cardiomyopathic aortas to phenylephrine is attributable to Ca^{2+} sensitization resulting from protein kinase C activation.

In summary and conclusion, our study demonstrated that the contractile responses to all vasoconstrictors tested were markedly enhanced in aortas from cardiomyopathic hamsters. The enhanced contractile responsiveness did not result from impairment of endothelial function, and occurred independently of alterations in Ca^{2+} handling by the endoplasmic reticulum or sarcolemma. The increased Ca^{2+} sensitivity of contractile apparatus can be considered to be mainly involved in the enhancement of vascular reactivity observed in cardiomyopathic hamsters.

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