ELSEVIER

European Journal of Pharmacology 318 (1996) 349–356

ejp

Force-frequency response in isoproterenol-induced hypertrophied rat heart

Lihui Tang [a,*], Weidong Gao [b], Paul B. Taylor [c]

[a] *Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6059, USA*
[b] *Division of Cardiology, Department of Medicine, School of Medicine at Johns Hopkins University, Baltimore, MD 21205, USA*
[c] *Department of Biological Sciences, University of Windsor, Windsor, Ontario, Canada N9B 3P4*

Received 9 May 1996; revised 13 August 1996; accepted 8 October 1996

Abstract

Rate-dependent force production was investigated using small trabecular muscle from control and hypertrophied rat cardiac muscle. Cardiac hypertrophy was induced by daily subcutaneous injection of isoproterenol (0.3 mg/kg body weight) for 12 days. The force-frequency relationship, for the control rat myocardium, is clearly biphasic. A stepped increase in stimulation frequency from 0.1 to 0.5 Hz results in a decrease in contractile force (negative phase). However, at higher stimulation frequency above 0.5 Hz, an increased contractile force is revealed (positive phase). Membrane action potential duration (APD_{50}) was used to reflect sarcolemmal Ca^{2+} influx. The frequency-dependent increase in APD_{50} and the ability of nifedipine, a sarcolemmal L-type Ca^{2+} channel blocker, to eliminate the positive-force frequency response, indicate that sarcolemmal Ca^{2+} influx is important for force development at high stimulation frequency. Relative Ca^{2+} content of sarcoplasmic reticulum is estimated from rapid cooling contractures. The parallel change of rapid cooling contractures and twitch force suggests that the sarcoplasmic reticulum Ca^{2+} content alters with varying frequencies of stimulation. Isoproterenol-induced hypertrophied muscle shows a greater contractile force, increased nifedipine-sensitive force development and prolonged APD_{50} compared to controls. These data suggest a greater availability of intracellular Ca^{2+} to activate contraction in hypertrophied muscle, possibly by amplified Ca^{2+} influx via L-type channel.

Keywords: Isoproterenol; Cardiac hypertrophy; Force-frequency response; (Rat); Ca^{2+}

1. Introduction

High serum levels of catecholamine are associated with cardiac hypertrophy (Boluyt et al., 1995; Lakatta, 1993; Morgan and Baker, 1991; Womble et al., 1980). Experimentally, cardiac hypertrophy can be induced by chronic exposure of isoproterenol, a selective β-adrenoceptor agonist. Although the detailed mechanisms of isoproterenol on induction of cardiac hypertrophy are unclear, there is evidence for a direct induction of isoproterenol on the cardiac growth process (Bishopric and Kedes, 1991; Simpson, 1985). Altered gene expression involved in both cell growth and differentiation to chronic isoproterenol exposure has been related to the development of myocyte hypertrophy (Boluyt et al., 1995; Slotkin et al., 1995). In this model of cardiac hypertrophy, an altered contractile function has been widely reported (Baldwin et al., 1982; Lakatta, 1993; Morgan and Baker, 1991; Tang and Taylor, 1996; Taylor et al., 1989). Normally, two major Ca^{2+}-dependent mechanisms regulate force production of the heart: the availability of intracellular Ca^{2+} ($[Ca^{2+}]_i$) to activate the myofilaments, and the sensitivity of the myofilaments to $[Ca^{2+}]_i$ (Morgan, 1991). Previous study in isoproterenol-induced cardiac hypertrophy shows unchanged responsiveness of the myofilaments to Ca^{2+} (force-pCa^{2+} relationship) in skinned trabecular muscle, suggesting that mechanisms associated with $[Ca^{2+}]_i$ transients may be selectively altered (Tang and Taylor, 1996).

The $[Ca^{2+}]_i$ transients are initiated when membrane depolarization activates Ca^{2+} entry through sarcolemmal L-type Ca^{2+} channels. Calcium influx triggers a large Ca^{2+} release from the sarcoplasmic reticulum. In rat cardiac muscle, most of activator $[Ca^{2+}]_i$ which activates the contractile apparatus originates from the sarcoplasmic reticulum (Bers, 1991; Nabauer and Morad, 1990). Accumulated evidence has shown that acute administration of

* Corresponding author. Tel./Fax: (1-610) 259-9493.

PII S0014-2999(96)00805-9

isoproterenol can increase the number of functioning L-type Ca^{2+} channels (Sperelakis, 1988) and prolong channel mean open time (Reuter et al., 1982). These combined functional responses will increase total Ca^{2+} conductance (Sperelakis, 1988). The focus of this study is to gain some insight into sarcolemmal Ca^{2+} influx and sarcoplasmic reticulum Ca^{2+} handling in the adaptation to chronic isoproterenol administration.

In rat cardiac muscle, the prevailing notion is that this species possesses either a unique negative or biphasic force-frequency relationship, in contrast to other mammalian species which exhibit positive rate-staircase responsiveness (Borzak et al., 1991; Orchard and Lakatta, 1985; Schouten and Ter Keurs, 1991; Stemmer and Tai, 1986). To our knowledge, the force-frequency relationship has not been reported for the isoproterenol-induced hypertrophied heart, therefore, the dependence of contractile force on beating rates is studied.

2. Materials and methods

2.1. Cardiac hypertrophy induction

Adult female Wistar rats weighing 200–250 g were used in this study. The female Wistar rats were chosen because the body weights of female rats are relatively constant compared to that of male rats. Therefore, it is reasonably accurate to evaluate the growth response of heart weight (e.g., expressed as heart weight/body weight) after isoproterenol administration. Cardiac hypertrophy was induced during 12 days of daily subcutaneous injections of isoproterenol (0.3 mg/kg body weight) suspended in sterilized olive oil (Taylor et al., 1989). Control animals received an equal volume of olive oil only. Animals were fasted 12 h before dissection. Tissue wet weight of the right ventricular free wall and the left ventricle plus septum was measured after removal of the large vessels, atria, and excess connective tissue. Dry weight was estimated after dehydrating the samples in an oven for 3 days at 60°C.

2.2. Muscle isolation and experimental condition

The isolation of trabecular muscles has been reported previously (Taylor et al., 1989). Briefly, hearts were removed under ether anesthesia. Thin, free running trabeculae were dissected between the atrial-ventricular valve and the right ventricular wall. The 3.0 mg/ml 3,3-butane dione monoxim was used to arrest contraction during isolation of trabecular muscles (Armstrong and Ganote, 1991). The similar size of trabecular muscle between control and hypertrophy (0.026 ± 0.002 mm^2 ($n = 22$) vs. 0.027 ± 0.006 mm^2 ($n = 26$)) was carefully chosen in this study. The isolated muscle was suspended horizontally between a force transducer (Akers 801 senso-nor, Norway) and a length adjustment device in a 0.5 ml muscle bath. The muscle bath solution contained the salts (mM): NaCl, 117.1; KCl, 5.0; $MgCl_2$, 1.2; Na_2SO_4, 2.4; NaH_2PO_4, 2.0; $NaHCO_3$, 27.0; glucose, 10.0 and pH = 7.4. Extracellular calcium ($[Ca^{2+}]_e$) was added from 1 M $CaCl_2$ stock solution to Ca^{2+}-free solution. The solution was oxygenated with 95% O_2 and 5% CO_2 and maintained at 26°C during the experiment. In drug studies, nifedipine was solubilized in 95% ethanol to provide a 1 mM stock solution. Samples were added directly from this stock solution to the perfusate to give a final 100 nM solution. The addition of nifedipine resulted in 0.01% (v/v) ethanol in the perfusate and had no effect on contractile force (Post et al., 1991). During the experiments the muscle and perfusate were protected from light to avoid nifedipine photodegradation. Ryanodine was added from 1 mM stock aqueous solution to give a final concentration of 100 nM. After the addition of these drugs, muscles were equilibrated for 30 min at 0.2 Hz before initiating the stimulation protocols. This period was adequate to provide a stable pharmacological effect on contractility.

Muscles were stimulated at specified rate(s) with a 4 ms pulse duration delivered through two platinum electrodes connected to a stimulator (Grass S6C). The average sarcomere spacing was measured by a video-camera system attached to the camera port of the inverted microscope. For each muscle, the cross-sectional area was estimated from width and thickness measured with an ocular micrometer. The contractile force was normalized by cross-section areas. For these studies the average diastolic sarcomere distance was adjusted so that the control and hypertrophied muscles were contracting at a resting sarcomere length of approximately 1.90 μm. We selected a shorter diastolic sarcomere spacing for a number of reasons: (1) Initial studies with hypertrophied muscles showed that, at a shorter diastolic length, muscles could maintain stable twitch contractions for 4–5 h and the muscle could withstand repeated rapid cooling contractures with no evidence of loss in contractile function. (2) The development of cardiac hypertrophy may result in changes of sarcomere length (shorter or longer). The altered sarcomere length will affect the comparison of contractile force between control and hypertrophy at the same sarcomere length. However, the systemic measurement of sarcomere length has eliminated the possibility of altered sarcomere length in hypertrophied muscle (Tang and Taylor, 1996), the contractile force can be compared between control and hypertrophy at a given sarcomere length.

2.3. Rapid cooling contractures

Rapid cooling of the trabecular muscles was achieved by switching perfusate solutions using solenoid valves (Cole Parmer) located close to the inlet of the muscle bath. The solenoid valves controlled the flow of a bypass system (either cold or warm) to the muscle bath. The exchange of

warm buffer (26°C) to cold (0–1°C) was typically completed in less than 0.4 s as measured by a thermocouple at the surface of the muscle.

2.4. Membrane action potential

Membrane action potential was measured using stepped flexible electrodes (Fedida et al., 1990). The electrode was filled with 3 M KCl and gave a resistance of 15–20 MΩ. A computer program (Unkelscope) was used to sample membrane action potentials (at 2 kHz). The peak amplitude and the duration of the action potential at 50% of its amplitude (APD_{50}) were routinely calculated using this program. Both the action potential and contractile force were measured simultaneously. Frequently, single cell impalement using the stepped electrode lasted several hours even if the muscle contracted vigorously at high stimulation frequencies.

2.5. Statistic analysis

Data were expressed as means ± standard error (S.E.). All comparisons were performed by either Student's *t*-test or multivaried analysis of variance (MANOVA) using a computer program (SYSTATW5).

3. Results

3.1. Induction of cardiac hypertrophy

Table 1 shows that repeated injection of low doses of isoproterenol can induce a significant degree of cardiac hypertrophy which is consistent with previous results (Tang and Taylor, 1996). In these experiments, animal body weight was similar between control and hypertrophied groups (219 g vs. 227 g). There was a 36% increase in the whole ventricle tissue mass in the isoproterenol-treated group compared to the controls. Since there was no evidence of increased tissue water content (79% vs. 81%, respectively, in control and hypertrophy), these hearts were significantly hypertrophied. Regionally, the right ventricle achieved a greater degree of hypertrophy than the left ventricle (47% vs. 30%).

Table 1
Chronic effects of isoproterenol on tissue mass

Parameters	Control	Hypertrophy	Increased ratio
WV/BW (mg/kg)	591.1 ± 3.3	802.3 ± 1.1 [a]	36%
LV/BW (mg/kg)	481.1 ± 1.9	631.3 ± 1.1 [a]	30%
RV/BW (mg/kg)	118.3 ± 0.9	173.8 ± 0.5 [a]	47%

[a] Significant difference from corresponding control value at $P < 0.001$. BW, body weight. WV, tissue dry weight of whole ventricle. LV, tissue dry weight of left ventricle. RV, tissue dry weight of right ventricle.

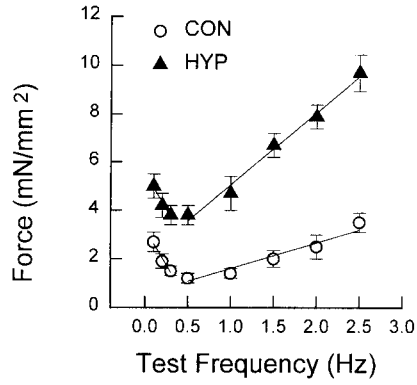

Fig. 1. Influence of stimulation rate on steady-state contractile force. Contractile force was normalized by muscle cross-sectional area. Significant differences were revealed throughout all test rates of stimulation between control (CON, $n = 10$) and hypertrophy (HYP, $n = 6$) (not labelled, $P < 0.001$). $[Ca^{2+}]_e = 0.5$ mM.

3.2. Force-rate relationship in hypertrophy

An increase in stimulation frequency caused a biphasic force-frequency response in both control and hypertrophied muscles (Fig. 1). A stepped increase in stimulation frequency from 0.1 to 0.5 Hz resulted in a decrease in contractile force (negative staircase). At frequencies above 0.5 Hz, there was clearly an enhanced contractile force (positive staircase) in both control and hypertrophied groups. The significantly increased contractile force from 0.5 Hz was found at 1.5 Hz for control ($P < 0.05$) and hypertrophied muscles ($P < 0.001$). However, at all frequencies studied, contractile force in hypertrophied muscle was significantly greater compared to the controls. During the positive phase of the force-frequency response, hypertrophied muscle showed significantly higher levels of force development with increasing frequencies of stimulation. To quantitatively reflect the amplified positive phase, the slopes of the positive phase were calculated from the best-fit lines of developed force vs. stimulation rate and showed that the slope was significantly greater in hypertrophied muscles than in controls (3.05 ± 0.53 ($r = 0.99$) vs. 1.03 ± 0.1 ($r = 0.95$), $P < 0.001$).

3.3. Effect of stimulation rates on the sarcoplasmic reticulum Ca^{2+} content

In rat cardiac muscle, most of activator Ca^{2+} which activates contraction originates from the sarcoplasmic reticulum (Bers, 1991; Nabauer and Morad, 1990). Rapid cooling of cardiac muscle can open sarcoplasmic reticulum Ca^{2+} release channel and release almost all of the releasable sarcoplasmic reticulum Ca^{2+} and, thus activates a contracture. The amplitude of the induced contracture is indicative of the releasable sarcoplasmic reticulum Ca^{2+} content (Bers and Christensen, 1990; Banijamali et al., 1991). Fig. 2 (top panel) shows an original recording of a typical rapid cooling contracture in control muscle which

is comparable to that previously reported (Bers and Christensen, 1990). Fig. 2 compares contractile force induced by electrical stimulation and rapid cooling contracture as a function of stimulation frequency. In order to reflect the sensitivity of both twitch force and the rapid cooling contracture to varied frequencies of stimulation, twitch force and rapid cooling contractures were respectively normalized by the minimal values at 0.5 Hz. In both control and hypertrophied muscles there was roughly a parallel change in twitch force and releasable sarcoplasmic reticulum Ca^{2+} content, suggesting that the releasable sarcoplasmic reticulum Ca^{2+} content was proportional to twitch tension. Also, the hypertrophied muscles showed a much more sensitive response of the rapid cooling contractures at the positive staircase. Although we cannot directly evaluate absolute sarcoplasmic reticulum Ca^{2+} content, a greater response of rapid cooling contractures in hypertrophied muscle during the positive phase suggests a relatively greater increase in the sarcoplasmic reticulum Ca^{2+} content with an increased stimulation frequency.

3.4. Effects of stimulation rate on action potential duration

The most consistent electrophysiological change in hypertrophied muscle is a prolonged duration of membrane action potential (Aronson, 1980; Bouron et al., 1992; Keung, 1989; Kleiman and Houser, 1988; Nordin et al., 1989; Scamps et al., 1990). APD_{50} was considered as an appropriate indicator of Ca^{2+} influx during action potential because: (1) 50% repolarization is at −30 mV to −20 mV so that APD_{50} covers the period of Ca^{2+} influx; (2) it has been clearly shown that the prolonged action potential duration with increasing frequency of stimulation can be eliminated by applying L-type Ca^{2+} blocker, nifedipine (Schouten and Ter Keurs, 1991); (3) in other models of hypertrophied rat heart, results of whole cell patch clamp have revealed that the prolonged action potential duration is due to the increased total number of L-type Ca^{2+} channels (Keung, 1989; Kleiman and Houser, 1988; Scamps et al., 1990).

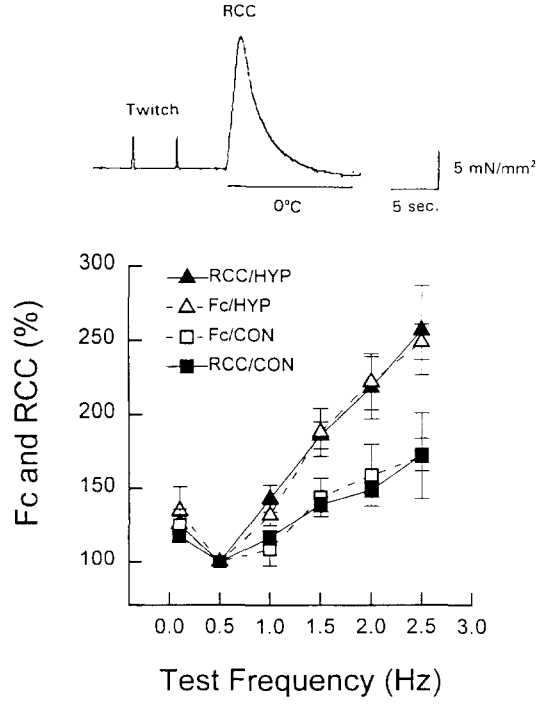

Fig. 2. Effect of stimulation rates on rapid cooling contracture (RCC) and contractile force (Fc) in both control (CON, $n = 7$) and hypertrophy (HYP, $n = 9$). All values of rapid cooling contracture and contractile force were normalized by the respectively minimal values at 0.5 Hz. The top inset shows an original recording of a rapid cooling contracture initiated at an interval of 5 s following last twitch contraction.

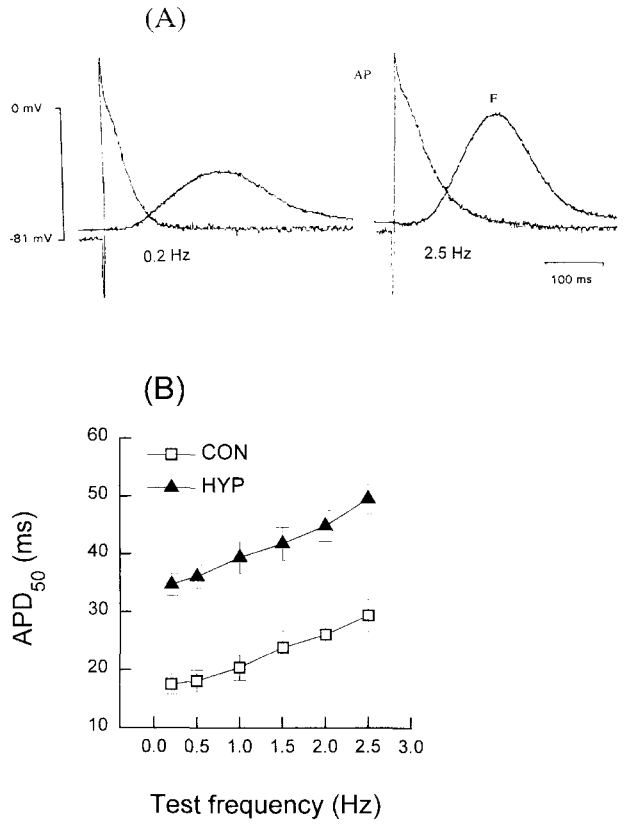

Fig. 3. Panel (A) shows the original recordings of action potential (AP) and twitch force (F), respectively, at 0.2 Hz and 2.5 Hz in control muscle. (B) The comparison of APD_{50} between control ($n = 6$) and hypertrophy ($n = 6$) at different rates of stimulation. The significantly increased APD_{50} in hypertrophy compared to control was revealed at all test rates (not labelled, MANOVA, $P < 0.05$).

A typical, simultaneous recording of membrane potential and contractile force at low (0.2 Hz) and high (2.5 Hz) stimulation rates is shown in Fig. 3A. In addition to the increase of contractile force at high frequency, careful examination of the action potentials revealed that the duration of action potential was prolonged. Fig. 3B shows the response of the APD_{50} as a function of stimulation rates. In both groups, there was a consistent lengthening of

the APD_{50} (MANOVA, $P < 0.01$) with increasing rate of stimulation. The absolute value of APD_{50} was significantly greater in hypertrophy compared with the controls at all frequencies of stimulation (MANOVA, $P < 0.05$).

3.5. Effects of Ca^{2+} transport modulators on force-rate response

In intact muscle, it has been shown that ryanodine, at nanomolar concentrations, can induce a Ca^{2+} leakage from the sarcoplasmic reticulum during diastole without influencing other mechanisms such as myofilament Ca^{2+} sensitivity, Na^{+}-Ca^{2+} exchanger and L-type Ca^{2+} channel function (Banijamali et al., 1991; Lynch, 1991). The contribution of sarcoplasmic reticulum Ca^{2+} loading during diastole on the biphasic force frequency response was explored by using 100 nM ryanodine. At this concentration, ryanodine can induce sarcoplasmic reticulum Ca^{2+} leakage, but still permit sarcoplasmic reticulum Ca^{2+} reuptake and release during electrically activated contractions (Banijamali et al., 1991). In the negative phase, ryanodine significantly decreased contractile force and transformed this response into a positive relation (Fig. 4) in both control (MANOVA, $P < 0.001$) and hypertrophied (MANOVA, $P < 0.01$) muscles. At frequencies above 0.5 Hz, ryanodine had no significant effect on the force-frequency response in either control or hypertrophied muscles. These results suggest that sarcoplasmic reticulum Ca^{2+} loading during diastole dominates the contractile force at low frequencies of stimulation. In hypertrophied muscle, the elevated contractile force (in the absence of ryanodine) at low stimulation frequencies may result from an enhanced sarcoplasmic reticulum Ca^{2+} loading. In the presence of ryanodine the hypertrophied muscles still developed greater force in all ranges of frequencies studied (Fig. 4), suggesting that other mechanisms, possibly sarcolemmal L-type Ca^{2+} channel (see below), are also amplified in hypertrophied muscles.

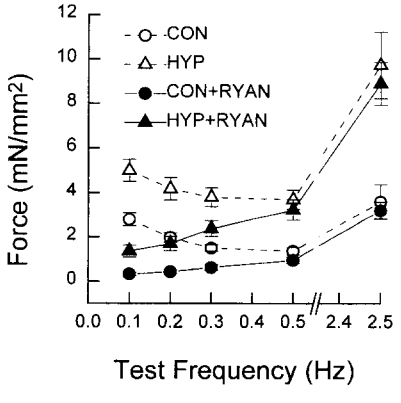

Fig. 4. Effect of ryanodine on the biphasic force-frequency response. There was a significant reduction of contractile force after ryanodine treatment at stimulation rates below 0.5 Hz in both control (MANOVA, $P < 0.001$) and hypertrophy (MANOVA, $P < 0.01$) (not labelled). CON: control ($n = 10$); CON+RYAN: ryanodine-treated control ($n = 6$); HYP: hypertrophy ($n = 6$); HYP+RYAN: ryanodine-treated hypertrophy ($n = 7$).

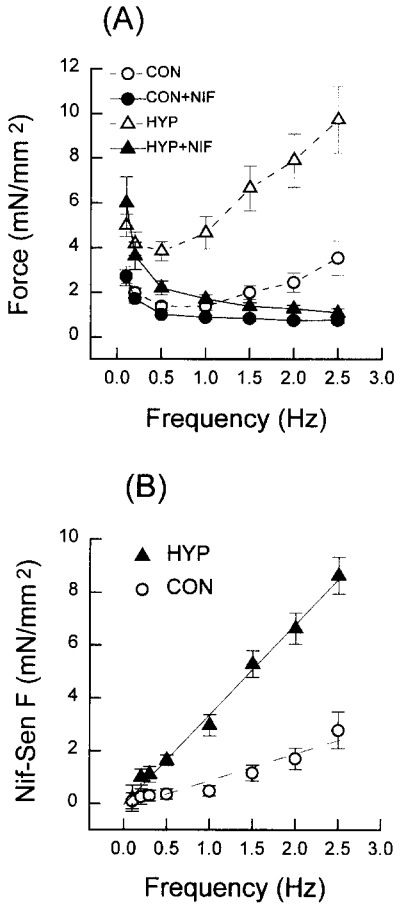

Fig. 5. (A) shows the effect of nifedipine on the biphasic force-frequency response. Nifedipine significantly decreased contractile force ($P < 0.05$) at stimulation rates either above 1.5 Hz in control or 0.5 Hz in hypertrophy (not labelled). CON: control ($n = 10$); CON+NIF: nifidipine-treated control ($n = 7$); HYP: hypertrophy ($n = 6$); HYP+NIF: nifidipine-treated hypertrophy ($n = 6$). (B) represents nifedipine-sensitive force development (Nif-Sen F). The nifedipine-sensitive force was calculated by comparing force difference before and after addition of nifedipine.

To determine the contribution of Ca^{2+} influx via sarcolemmal L-type Ca^{2+} channel on the biphasic response, we exposed muscles to 100 nM nifedipine, which is known to block L-type Ca^{2+} channel conductance (Post et al., 1991; Schouten and Ter Keurs, 1991). In contrast to ryanodine, nifedipine had little effect at low stimulation rates (Fig. 5A). At frequencies greater than 0.5 Hz, nifedipine decreased contractile force and transformed the positive response to a negative relation in both the control and hypertrophied muscles. Nifedipine-sensitive force development was estimated by comparing force difference before and after addition of nifedipine at each test rate. The different nifedipine-sensitive contractile force for control and hypertrophied muscles at each test frequency is shown in Fig. 5B. Clearly, there was a significantly greater nifedipine-sensitive contractile force in hypertrophied muscles in the positive force-frequency range, suggesting that

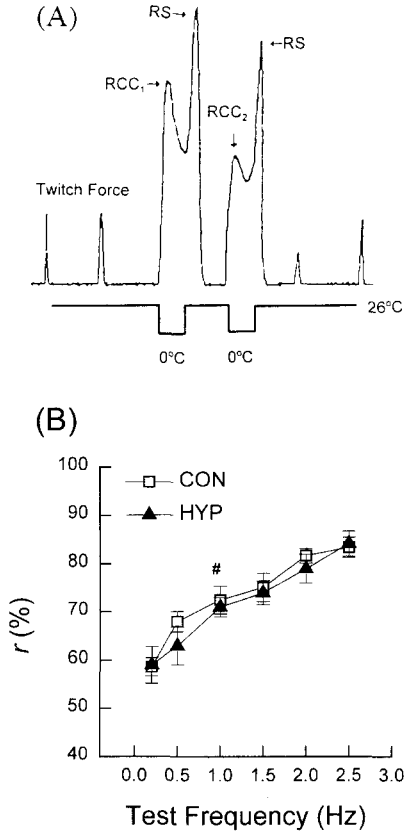

Fig. 6. The recirculation fraction of sarcoplasmic reticulum Ca^{2+}, 'r', evaluated by paired rapid cooling contractures. (A) shows an example of original recordings at a stimulation rate of 0.2 Hz in control muscle. The first rapid cooling contracture (RCC_1) was initiated at 5 s intervals following last twitch force, rewarmed, then recooled (RCC_2) and rewarmed again. RS: rewarming spike. (B) shows 'r' (RCC_2/RCC_1) as a function of stimulation rate. There was no difference in 'r' between control ($n = 7$) and hypertrophy ($n = 6$). '#' represents increased 'r' ($P < 0.05$) at stimulation rates above 1.0 Hz compared to values at 0.1 Hz in both groups.

Ca^{2+} influx via sarcolemmal L-type Ca^{2+} channels became amplified during the development of cardiac hypertrophy.

3.6. Recirculation fraction of the sarcoplasmic reticulum Ca^{2+}

The double rapid cooling contractures were used to evaluate the fraction of sarcoplasmic reticulum Ca^{2+} sequestration following a contraction (Hryshko et al., 1989). A typical recording of paired rapid cooling contractures is shown in Fig. 6A. Rapid cooling of the muscle induces sarcoplasmic reticulum Ca^{2+} release to the cytoplasm and subsequently activates a rapid contracture (RCC_1). The amplitude of rapid cooling contracture has been used as a relative index of releasable sarcoplasmic reticulum Ca^{2+} content (Bers and Christensen, 1990; Banijamali et al., 1991). Rapid cooling is neither accompanied by an action potential (no electrical stimulation is applied) nor by a depolarization of sufficient magnitude to activate ionic channels, therefore, no extracellular Ca^{2+} influx is involved in the rapid cooling contracture. When the muscle was quickly rewarmed to 26°C, mechanisms responsible to remove cytoplasmic Ca^{2+}, such as sarcoplasmic reticulum Ca^{2+} resequestration via Ca^{2+}-ATPase and Ca^{2+} extrusion via sarcolemmal Na^{+}-Ca^{2+} exchanger, were reactivated. During rewarming, there was a transient increase in force (rewarming spike). The rewarming spike was due to the rapid recovery of myofilament Ca^{2+} sensitivity during rewarming. The second rapid cooling contracture (RCC_2) was initiated at a time lag less than 3 s after the first rapid cooling contracture was rewarmed and returned to complete relaxation. Because rewarming of the first rapid cooling contracture to the complete relaxation represents the removal of cytoplasmic Ca^{2+} (released from the sarcoplasmic reticulum during the first rapid cooling contracture) via either sarcoplasmic reticulum Ca^{2+}-ATPase pump or Na^{+}-Ca^{2+} exchanger, the ratio of the second rapid cooling contracture normalized by the first rapid cooling contracture provides a relative index of the amount of Ca^{2+} resequestered by the sarcoplasmic reticulum during the rewarming of the first rapid cooling contracture.

The response of sarcoplasmic reticulum Ca^{2+} recirculation fraction, 'r' (RCC_2/RCC_1), at various frequencies of stimulation was analyzed in both control and hypertrophied muscles (Fig. 6B). Analysis of the 'r' shows that (1) there is no difference in the relative 'r' between control and hypertrophied muscles; (2) the 'r' is sensitive to stimulation frequency and is significantly enhanced above 1.0 Hz compared to the values at 0.2 Hz in both control and hypertrophied muscles ($P < 0.05$); (3) most of released sarcoplasmic reticulum Ca^{2+} at the first rapid cooling contracture was resequestered by the sarcoplasmic reticulum during rewarming, because the second rapid cooling contracture produced amplitudes of 58.4–83.4% and 58.6–84.2% of the first rapid cooling contracture, respectively, in control and hypertrophied muscles at stimulation rates from 0.2 Hz to 2.5 Hz.

4. Discussion

4.1. Altered Ca^{2+} handling in hypertrophied muscle

In general, a change in peak force can be explained by either a diminished or an enhanced sarcoplasmic reticulum Ca^{2+} release in rat myocardium (Bers, 1991; Morgan, 1991). In this study, the possible involvement of altered contractile proteins on the contractile force in hypertrophy can be excluded based on our previous force-pCa^{2+} results in which there is an unchanged sensitivity of myofilaments to Ca^{2+} (Tang and Taylor, 1996). Therefore, the contractile force is believed to reflect free $[Ca^{2+}]_i$ concentrations. The loading of sarcoplasmic reticulum Ca^{2+} for contraction is from two primary sources: the Ca^{2+} influx via sarcolemmal L-type Ca^{2-} channel and some contribution

by sarcolemmal Na^+-Ca^{2+} exchanger at long resting intervals, and $[Ca^{2+}]_i$ recirculation between the myoplasm and the sarcoplasmic reticulum during the relaxation phase (Bers and Christensen, 1990). To address whether the magnitude of the steady-state peak force is proportional to the amount of free $[Ca^{2+}]_i$ released from the sarcoplasmic reticulum at various stimulation rates, we took advantage of applying rapid cooling contractures to evaluate the sarcoplasmic reticulum Ca^{2+} release. We reasoned that if sarcoplasmic reticulum Ca^{2+} loading contributed to developed force then the rapid cooling contracture should show different changes with altered frequencies of stimulation. In this study, twitch force and the rapid cooling contracture changed in parallel with varied stimulation frequencies. These data argue, that: (1) at all stimulation frequencies the releasable sarcoplasmic reticulum Ca^{2+} is proportional to the electrically stimulated twitch force in hypertrophied muscles, and (2) in the hypertrophied muscle there is a relatively greater increase of the sarcoplasmic reticulum Ca^{2+} with increasing stimulation rate at the positive phase.

The paired rapid cooling contractures (Fig. 6) revealed that there was a significant dependence of recirculation fraction of the sarcoplasmic reticulum Ca^{2+} on the stimulation rate in both control and hypertrophied muscles. The behavior can be explained on the basis of competitive mechanism for cytoplasmic Ca^{2+} removal between the sarcoplasmic reticulum Ca^{2+}-ATPase pump and sarcolemmal Na^+-Ca^{2+} exchanger. Normally, there is an increase in intracellular Na^+ concentration with increasing rate of stimulation due to more action potentials per unit time (Schouten and Ter Keurs, 1991). An increase in intracellular Na^+ concentration will inhibit Ca^{2+} efflux via Na^+-Ca^{2+} exchanger, which favors the competition of the sarcoplasmic reticulum Ca^{2+}-ATPase pump and thus increases the recirculation fraction of sarcoplasmic reticulum Ca^{2+} with increasing stimulation rate.

A biphasic force-frequency relationship of rat myocardium was revealed in this study which is consistent with others using isolated trabeculae (Schouten and Ter Keurs, 1991) or myocytes (Borzak et al., 1991), suggesting that two different mechanisms are involved in the force development at different stimulation rates. In hypertrophied muscle, the force development was increased throughout all rates and especially, a much more sensitive increase of contractile force was shown in the positive phase. This implied amplified $[Ca^{2+}]_i$ transients, especially at the positive phase in hypertrophied muscle.

4.2. Negative phase

In both hypertrophied and control muscles, ryanodine-treated muscles removed the negative phase and revealed only a positive force-frequency response. These results support the notion that during low stimulation rates and/or in the rest-potentiated state, which is well developed in the rat, the negative rate staircase depends primarily on relatively avid sarcoplasmic reticulum Ca^{2+} loading due to Ca^{2+} influx, possibly via Na^+-Ca^{2+} exchanger (Bers and Bridge, 1989; Borzak et al., 1991; Orchard and Lakatta, 1985; Schouten, 1990; Schouten and Ter Keurs, 1991; Stemmer and Tai, 1986). We believe that the Na^+-Ca^{2+} exchanger mainly contributes to Ca^{2+} influx and loads the sarcoplasmic reticulum at lower stimulation frequencies based on the following reasons: (1) In the rat, intracellular Na^+ is high enough (12.7 mM) to induce Ca^{2+} influx via the Na^+-Ca^{2+} exchanger, especially at the longer rest intervals (Bers and Christensen, 1990). (2) Ca^{2+}-sensitive microelectrode data clearly show a rest induced transsarcolemmal Ca^{2+} influx (Shattock and Bers, 1989). (3) Since we blocked the L-type Ca^{2+} channels, one of the two major Ca^{2+} influx pathways, developed force still remained unchanged at low contraction rates. In the hypertrophied muscles, the greater contractile force at negative phase would suggest an amplified Ca^{2+} influx via the Na^+-Ca^{2+} exchanger. At low stimulation frequencies, if the sarcoplasmic reticulum Ca^{2+} loading dominates force development, this study shows that the ryanodine-treated hypertrophied muscle in which sarcoplasmic reticulum Ca^{2+} was unloaded still produced greater contractile force than the controls (Fig. 4). One of the explanations is that the increased Ca^{2+} influx via L-type Ca^{2+} channel, as revealed by an increased APD_{50} throughout all stimulation rates, contributes the greater contractile force in the ryanodine-treated hypertrophied muscle. Although the contribution of Ca^{2+} influx via L-type channel to contractile force is little at low stimulation rates (Fig. 5A), the amplified Ca^{2+} influx could result in the greater contractile force in hypertrophied muscles under conditions of ryanodine treatment in which a diminished contribution of sarcoplasmic reticulum Ca^{2+} release exists.

4.3. Positive phase

Recent studies have proposed that Ca^{2+} influx via sarcolemmal L-type channels contributes force production at high stimulation rates in rat myocardium (Borzak et al., 1991; Schouten and Ter Keurs, 1991). In the nifedipine-treated muscles, contractile force was significantly diminished and the positive phases were converted into negative relations in both groups. In this study, the frequency-induced increase of Ca^{2+} influx can be also supported by the consistently prolonged APD_{50}. This prolonged APD_{50} in isoproterenol-induced cardiac hypertrophy is consistent with previous results reported by Meszaros (1992). The greater absolute value of APD_{50} in hypertrophy at all stimulation rates suggests an enhanced Ca^{2+} influx via L-type Ca^{2+} channels. Also, in hypertrophied muscle, a significantly enhanced nifedipine-sensitive force development was revealed with increasing stimulation frequency (Fig. 5B). Therefore, the greater contractile force in hypertrophied muscles results from the amplified Ca^{2+} influx via L-type Ca^{2+} channels.

In summary, a biphasic force-frequency responsiveness is revealed in both control and hypertrophied muscles. Hypertrophied muscle showed a greater contractile force and a parallel enhancement of the relative sarcoplasmic reticulum Ca^{2+} content with various stimulation rates, suggesting a higher $[Ca^{2+}]_i$ available for activating contraction. The amplified positive phase, nifedipine-sensitive force development, and prolonged APD_{50} with increasing frequency of stimulation suggest an enhanced Ca^{2+} influx via sarcolemmal L-type Ca^{2+} channels in cardiac hypertrophy induced by isoproterenol.

Acknowledgements

The authors thank Dr. Henk E.D.J. Ter Keurs, University of Calgary in Canada, for excellent technical assistance in making the flexible microelectrodes.

References

Armstrong, S.C. and C.E. Ganote, 1991, Effect of 2,3-butanedione monoxime on contracture and injury of isolated rat myocyte following metabolic inhibition and ischemia, J. Mol. Cell. Cardiol. 23, 1001.

Aronson, R.S., 1980, Characteristics of action potentials of hypertrophied myocardium from rats with renal hypertension, Circ. Res. 47, 443.

Baldwin, K.M., S.B. Ernst, W.I. Mullin, L.F. Schrader and R.E. Herrick, 1982, Exercise capacity and cardiac function of rats with drug-induced cardiac enlargement, J. Appl. Physiol. 52, 591.

Banijamali, H.S., W.D. Gao, B.R. Macintosh and H.E.D.J. Ter Keurs, 1991, Force-interval relations of twitch and cold contractures in rat cardiac trabeculae, Circ. Res. 69, 937.

Bers D.M., 1991, Excitation-Contraction Coupling and Cardiac Force (Kluwer Academic Publishers, Dordrecht) p. 148.

Bers, D.M. and J.H.B. Bridge, 1989, Relaxation of rabbit ventricular muscle by Na^+-Ca^{2+} exchange and sarcoplasmic reticulum Ca^{2+}-pump: ryanodine and voltage sensitivity, Circ. Res. 65, 334.

Bers, D.M. and D.M. Christensen, 1990, Functional interconversion of rest decay and ryanodine effects in rabbit and rat ventricle depends on Na^+-Ca^{2+} exchanger, J. Mol. Cell. Cardiol. 22, 715.

Bishopric, N. and L. Kedes, 1991, Adrenergic regulation of the skeletal alpha-actin gene promotor during myocardial cell hypertrophy, Proc. Natl. Acad. Sci. USA 88, 2132.

Boluyt, M.O., X.L. Long, T. Eschenhagen, U. Mende, W. Schmitz, M.T. Crow and E.G. Lakatta, 1995, Isoproterenol infusion induces alterations in expression of hypertrophy-associated genes in rat-heart, Am. J. Physiol. 269, H638.

Borzak, S., M. Stephanie and J.D. Marsh, 1991, Mechanisms of rat staircase in rat ventricular cells, Am. J. Physiol. 260, H884.

Bouron, A., D. Potreao and G. Raymond, 1992, The L-type Ca^{2+} current in single hypertrophied cardiac myocytes isolated from right ventricle of ferret heart, Cardiovasc. Res. 26, 662.

Fedida, D., S. Sethi, B.J.M. Mulder and H.E.D.J. Ter Keurs, 1990, An ultracompliant glass microelectrode for intracellular recording, Am. J. Physiol. 258, C164.

Hryshko, L.V., V. Stiffel and D.M. Bers, 1989, Rapid cooling contractures as an index of sarcoplasmic reticulum calcium content in rabbit ventricular myocytes, Am. J. Physiol. 257, H1369.

Keung, E.C., 1989, Calcium current is increased in isolated adult myocytes from hypertrophied rat myocardium, Circ. Res. 64, 753.

Kleiman, R.B. and S.R. Houser, 1988, Calcium currents in normal and hypertrophied isolated feline ventricular myocytes, Am. J. Physiol. 255, H1434.

Lakatta, E.G., 1993, Cardiovascular regulatory mechanisms in advanced age, Physiol. Rev. 73, 413.

Lynch III, C., 1991, Pharmacological evidence for two types of myocardial sarcoplasmic reticulum calcium release, Am. J. Physiol. 260, H785.

Meszaros, J., 1992, Sodium pump injury and arrythmogenic transient depolarizations in catecholamine-induced cardiac hypertrophy, Eur. J. Pharmacol. 210, 325.

Morgan, H.E. and K.M. Baker, 1991, Cardiac hypertrophy, Circulation 1, 13.

Morgan, J.P., 1991, Abnormal intracellular modulation of calcium as a major cause of cardiac contractile dysfunction, New Engl. J. Med. 325, 625.

Nabauer, M. and M. Morad, 1990, Ca^{2+} induced Ca^{2+} release as examined by photolysis of caged Ca^{2+} in single ventricular myocytes, Am. J. Physiol. 258, C189.

Nordin, C., F. Siri and R.S. Aronson, 1989, Electrophysiological characteristics of single myocytes isolated from hypertrophied guinea-pig hearts, J. Mol. Cell. Cardiol. 21, 729.

Orchard, C.H. and E.G. Lakatta, 1985, Intracellular calcium transients and developed tension in rat heart muscle. A mechanism for the negative interval strength relationship, J. Gen. Physiol. 86, 637.

Post, J.A., J.I. Sen, S.L. Kenneth and G.A. Langer, 1991, Effects of charged amphiles on cardiac cell contractility are mediated via effects on calcium current, Am. J. Physiol. 260, H759.

Reuter, H., C.F. Stevens, R.W. Tsien and G. Yellen, 1982, Properties of single calcium channels in cardiac cell culture, Nature 297, 501.

Scamps, F., E. Mayoux, D. Charlemagne and G. Vassort, 1990, Calcium current in single cells isolated from normal and hypertrophied rat heart, Circ. Res. 67, 199.

Schouten, V.J.A., 1990, Interval dependence of force and twitch duration in rat heart explained by calcium pump inactivation in the sarcoplasmic reticulum, J. Physiol. 431, 427.

Schouten, V.J.A. and H.E.D.J. Ter Keurs, 1991, Role of I_{Ca} and Na^+-Ca^{2+} exchange in the force-frequency relationship of rat heart muscle, J. Mol. Cell. Cardiol. 23, 1039.

Shattock, M.J. and D.M. Bers, 1989, Rat vs. rabbit ventricle: calcium flux and intracellular sodium by ion selective microelectrodes, Am. J. Physiol. 256, C813.

Simpson, P., 1985, Stimulation of hypertrophy of cultured neonatal rat heart cells through an alpha1-adrenergic receptor and induction of beating through an alpha1- and beta1-adrenergic receptor interaction, Circ. Res. 56, 884.

Slotkin, T.A., S.E. Lappi and F.J. Seidler, 1995, Beta-adrenergic control of C-fos expression in fetal and neonatal rat tissues: relationship to cell-differentiation and teratogenesis, Toxicol. Appl. Pharmacol. 133, 188.

Sperelakis, N., 1988, Regulation of calcium slow channels of cardiac muscle by cyclic nucleotides and phosphorylation, J. Mol. Cell. Cardiol. 20 (Suppl. 2), 75.

Stemmer, P. and A. Tai, 1986, Concealed positive-force-frequency relationships in rat and mouse cardiac muscle by ryanodine, Am. J. Physiol. 251, H1106.

Tang, L.H. and P.B. Taylor, 1996, Altered contractile function in isoproterenol induced hypertrophied rat heart, J. Hypertens. 14, 751.

Taylor, P.B., R.K. Helbin, S. Rourke and D. Churchill, 1989, Effects of catecholamine-induced cardiac hypertrophy on the force-interval relationship, Can. J. Physiol. Pharmacol. 67, 40.

Womble, J.R., D.F. Larson, J.G. Copeland, B.R. Brown, M.K. Haddox and D.H. Russell, 1980, Adrenal medulla denervation prevents stress-induced epinephrine plasma elevation and cardiac hypertrophy, Life Sci. 27, 2417.