Mibefradil Improves β-Adrenergic Responsiveness and Intracellular Ca^{2+} Handling in Hypertrophied Rat Myocardium

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The present study investigated the effects of mibefradil, a novel T-type channel blocker, on ventricular function and intracellular Ca^{2+} handling in normal and hypertrophied rat myocardium. Ca^{2+} transient was measured with the bioluminescent protein, aequorin. Mibefradil (2 μ*M*) produced nonsignificant changes in isometric contraction and peak systolic intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in normal rat myocardium. Hypertrophied papillary muscles isolated from aortic-banded rats 10 weeks after operation demonstrated a prolonged duration of isometric contraction, as well as decreased amplitudes of developed tension and peak Ca^{2+} transient compared with the sham-operated group. Additionally, diastolic $[Ca^{2+}]_i$ increased in hypertrophied rat myocardium. The positive inotropic effect of isoproterenol stimulation was blunted in hypertrophied muscles despite a large increase in Ca^{2+} transient amplitude. Afterglimmers and corresponding aftercontractions were provoked with isoproterenol (10^{-5} and 10^{-4} *M*) stimulation in 4 out of 16 hypertrophied muscles, but were eliminated in the presence of mibefradil (2 μ*M*). In addition, hypertrophied muscles in the presence of mibefradil had a significant improvement of contractile response to isoproterenol stimulation and a reduced diastolic $[Ca^{2+}]_i$, although a mild decrease of peak Ca^{2+}-transient was also shown. However, verapamil (2 μ*M*) did not restore the inotropic and Ca^{2+} modulating effects of isoproterenol in hypertrophied myocardium. Mibefradil partly restores the positive inotropic response to β-adrenergic stimulation in hypertrophied myocardium from aortic-banded rats, an effect that might be useful in hypertrophied myocardium with impaired $[Ca^{2+}]_i$ homeostasis. [Exp Biol Med Vol. 227(5):336–344, 2002]

Key words: hypertrophied myocardium; intracellular Ca^{2+}; mibefradil

Calcium antagonists are used widely in treatment of hypertension (1, 2) and angina pectoris (3). These agents belong to three main chemical classes: dihydropyridines (nifedipine), phenylalkylamines (verapamil), and benzothiazepines (diltiazem). Limitations of these traditional calcium antagonists include limited bioavailability, negative inotropy, reflex tachycardia, sympathetic stimulation, neurohormonal activation, and depression of atrioventricular nodal conduction (4). Mibefradil, a tetralol derivative, is the first of a new class of calcium antagonists that has been proved to decrease heart rate and dilate cardiac vascular beds, but without a negative inotropic effect (5). Unlike other available calcium antagonists, mibefradil selectively blocks low voltage-gated T-type calcium channels (5, 6). The effect of calcium influx inhibition induced by mibefradil is 10 times more powerful for T-type calcium channels than for low voltage-gated slow calcium channels; i.e., L-calcium channels (6).

T-type Ca^{2+} channels are abundant in the fetal heart (7). With postnatal development, the presence of this class of Ca^{2+} channel decreases in a progressive manner. The density of T-type Ca^{2+} channels is predominant only in pacemaker cells of the sinoatrial node and the Purkinje fibers (8, 9), and is rarely expressed in rat cardiac muscle during maturation (10). Recently, re-expression of T-type Ca^{2+} channels has been found in post-myocardial infarction and remodeled myocytes (11), genetic cardiomyopathy hearts (12), and hypertrophied rat hearts induced by aortic banding (13). Nuss and Houser (14) reported a T-type Ca^{2+} channel re-expression during hypertrophy in adult feline ventricle. However, the purpose of increased T-type Ca^{2+} channels in diseased hearts is not clearly understood.

Previous studies in our laboratory (15) showed evidence of altered intracellular Ca^{2+} handling in hypertrophied ferret cardiac muscle. Subsequently, we observed a blunted positive inotropic response to isoproterenol stimulation in papillary muscles isolated from hypertrophied rats after long-term pressure overload (16, 17). The abnormal calcium handling and excitation-contraction uncoupling

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might be associated with re-expression of T-type Ca^{2+} channels, consequently inducing Ca^{2+} overload and negative inotropic effects to β-adrenergic responsiveness in hypertrophied myocardium. The present study was designed to test the effects of mibefradil, a special T-type Ca^{2+} channel antagonist, compared with verapamil, on cardiac contractility and intracellular Ca^{2+} handling in normal and hypertrophied myocardium.

Materials and Methods

Animals. Male Lewis rats (Charles River Laboratories, Wilmington, MA) (age, 3 months; body weight, 210–250 g) were used in the present study. Animals were kept under climate-controlled conditions with a 12:12-hr light: dark cycle and they were provided with standard rat chow and tap water *ad libitum*. All experiments were performed with The Guiding Principles in the Care and Use of Animals according to the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Endotracheal intubation was performed and was followed by artificial ventilation under anesthesia with ether. Subsequently, anesthesia was maintained with intravenous methohexital sodium given via the tail vein during the procedure. The chest was opened by right side thoracotomy. The aortic stenosis was produced using a 12-gauge needle of 1.2 mm external diameter tied tightly together with a surgical thread. The needle was then rapidly removed leaving the ascending aorta constricted to a diameter of 1.2 mm. Thoracic cavity, muscle, and skin were then sutured separately. All rats were maintained on standard rat chow and water for 10 weeks after the operation.

Isolated Muscle Performance. Ten weeks after surgery, the rats were sacrificed during pentobarbital deep anesthesia. The heart was rapidly excised and placed in a dissecting chamber containing a modified Krebs-Henseleit solution with the following composition (in millimoles): NaCl 120, KCl 5.9, dextrose 5.5, $NaHCO_3$ 25, NaH_2PO_4 1.2, $MgCl_2$ 1.2, and $CaCl_2$ 1.0, pH 7.4, bubbled with carbogen (a mixture of 95% O_2 and 5% CO_2) at room temperature. Left ventricular papillary muscle was carefully dissected and then fixed to a muscle holder with a spring clip. The muscle preparations were then mounted in a 50-ml tissue bath containing modified Krebs-Henseleit solution maintained at 30°C and continuously bubbled with carbogen. The measurement of isometric contraction in papillary muscle was performed by a method described previously (17, 18). The following parameters of isometric contraction were recorded from each muscle: developed tension (DT, tension produced by the stimulated muscle), time to peak tension (TPT, time from the beginning of contraction to peak tension), and time to 50% relaxation (RT_{50}, time from peak tension to 50% of relaxation). At the end of the experiment, muscle preparations were 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 isolating the papillary muscle for study, the weights of the right and left ventricle (including the septum) were normalized by body weight and used as an index of hypertrophy.

Aequorin Light Signal Measurement. Aequorin (Friday Harbor Laboratory, WA) was loaded into muscle preparations by the macroinjection technique (17, 18). Aequorin (1–2 μl, 2 mg/ml) was injected briefly under the epimysium at the base of the muscle with a short-glass micropipette. After equilibration for a 90- to 120-min period while steady state was reached, stimulation was restarted at 0.33 Hz. The aequorin light signal was detected with the method previously described (17, 18). Parameters derived from the light signals were recorded in each muscle preparation, including the amplitude of transient, time to peak light (TPL) and time from peak to 50% fall in peak light (RL_{50}). The free intracellular concentration of calcium ($[Ca^{2+}]_i$) was estimated by normalizing the recorded light signal during isometric twitch by the maximal amount of light emitted after lysis of muscle membranes at the end of the experiment. Lysis was achieved with a 5% solution of the detergent Triton X-100 in phosphate-free physiological salt solution containing 50 m*M* Ca^{2+}. The normalized light signal was then converted to $[Ca^{2+}]_i$ using an *in vitro* calibration curve as described previously (17, 18).

Calcium and Isoproterenol Dose-Response Determinations. After obtaining baseline parameters, mibefradil (2 μ*M*) or verapamil (2 μ*M*) was added into the bath solution. Measurements of steady-state condition with these additional drugs were performed after 15 min. Phosphate was removed from the bath to avoid the possibility of precipitation at a higher concentration of extracellular Ca^{2+} ($[Ca^{2+}]_o$). The steady-state response to each change of $[Ca^{2+}]_o$ (0.5, 1.0, 2.0, 3.0, and 4.0 m*M*) was recorded at the plateau of inotropic response, which was reached after 10 min. The bath solution was then switched back to modified Krebs-Henseleit solution containing 1.0 m*M* Ca^{2+}. Steady-state conditions were observed again for 15 min. Mibefradil (2 μ*M*) or verapamil (2 μ*M*) was re-added into the bath solution. Fifteen minutes later, isoproterenol (10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} *M*) was added cumulatively to determine inotropic response to β-adrenergic stimulation in the presence of 2 μ*M* mibefradil or 2 μ*M* verapamil. Light signals and isometric contractions were measured 10 min after each dose of isoproterenol. Calcium and isoproterenol dose-responses were performed on the following study groups: Sham-Control, papillary muscles isolated from sham-operated rats 10 weeks after surgery without drug; Sham-Mibefradil, papillary muscles isolated from sham-operated rats 10 weeks after surgery in the presence of mibefradil; LVH-Control, hypertrophied muscles isolated from aortic-banded rats 10 weeks after surgery without drug; LVH-Mibefradil, hypertrophied muscles isolated from aortic-banded rats 10 weeks after surgery in the presence of mibefradil, and LVH-Verapamil, hypertrophied muscles isolated

from aortic-banded rats 10 weeks after surgery in the presence of verapamil. Each group consisted of eight muscle preparations.

Statistical Analysis. Data are presented as mean ± SD. Compared statistical significance for independent evaluations over all groups was determined by one-way analysis of variance (ANOVA). A repeated ANOVA was used for the same group that was subjected to extracellular calcium or isoproterenol stimulation. Unpaired Student's *t* test with Bonferroni correction was applied to analyze between-group comparison. $P < 0.05$ was considered statistically significant.

Results

Forty successfully operated rats were studied, including 24 rats that underwent banding of the ascending aorta and 16 rats that received a sham operation. Ten weeks after aortic banding, left ventricular weight and the ratio of left ventricular weight to body weight were increased (Table I). The cross-sectional area in papillary muscles isolated from aortic-banded rats 10 weeks after operation was remarkably increased compared with the sham-operated rats. The body weight and the ratio of right ventricular weight to body weight were similar in all groups. Table II shows the data of mechanical properties and intracellular Ca^{2+} transients determined from aequorin light signal of each group at baseline condition. Hypertrophied muscles isolated from aortic-banded rats showed a significant decrease in DT, and prolongation of time courses in contraction and relaxation compared with the sham-operated group. TPL and RL_{50} were also prolonged in hypertrophied muscles (Table II). Increased DT and peak systolic $[Ca^{2+}]_i$ in response to increased $[Ca^{2+}]_o$ are displayed in Figure 1. Mibefradil produced nonsignificant effects in contractility and peak systolic $[Ca^{2+}]_i$ in papillary muscles isolated from sham-operated rats. The inotropic effect to an increase of extracellular Ca^{2+} was similar in papillary muscles isolated from sham-operated rats with or without mibefradil. The concentration-curve to extracellular Ca^{2+} dose-response in the hypertrophied control group was shifted downward ($P < 0.05$) compared with the sham-operated group. The increase of amplitudes of isometric tension and Ca^{2+} transient to extracellular Ca^{2+} stimulation in the hypertrophied muscle group was blunted in the presence of verapamil. After washout of 4.0 m*M* $[Ca^{2+}]_o$ and replacement with Krebs-Henseleit solution containing 1.0 m*M* $[Ca^{2+}]_o$, DT showed no significant changes and was restored close to baseline values in papillary muscles of all groups (Fig. 1).

A graded increase in the concentration of isoproterenol resulted in a significant increase of DT in papillary muscles isolated from sham-operated rats in parallel with an increase of $Ca^{2+}{}_i$ transients (Figs. 2 and 3). This positive tension response was markedly blunted in papillary muscles isolated from aortic-banded rats without additional drug treatment despite an increase in peak amplitude of the $Ca^{2+}{}_i$ transient (Figs. 3 and 4). DT showed a nonsignificant reduction in cardiac muscles isolated from sham-operated and aortic-banded rats in the presence of mibefradil. With additional verapamil, the decrease in DT was significantly larger in hypertrophied papillary muscles than in mibefradil-treated muscle preparations isolated from aortic-banded rats (Figs. 3 and 4). The similar change in peak systolic $[Ca^{2+}]_i$ in each group in the presence of mibefradil or verapamil is shown in Figures 3 and 4. Lack of inotropic response to isoproterenol stimulation was similar in hypertrophied muscles isolated from aortic-banded rats in the presence of verapamil compared with the hypertrophied control group (Figs. 3 and 4).

The representative recordings (Fig. 2) demonstrated parallel increases in $[Ca^{2+}]_i$ and isometric force to isoproterenol stimulation in papillary muscles isolated from sham-operated rats. In contrast, increase of $[Ca^{2+}]_i$ in muscle preparations isolated from aortic-banded rats without additional drug treatment did not produce a corresponding increase in developed tension (Fig. 4A). Additional mibefradil resulted in a significantly improved inotropic response to isoproterenol in hypertrophied muscles compared with that of muscle preparations isolated from 10 week aortic-banded rats without additional mibefradil treatment and in the presence of verapamil, although $Ca^{2+}{}_i$ availability in the presence of mibefradil was less than that of hypertrophied con-

Table I. General characteristics of sham-operated and aortic-banded rat hearts

	BW (g)	LVW (mg)	RVW (mg)	LVW/BW (mg/g)	RVW/BW (mg/g)	CSA (mm^2)
Sham-I	432 ± 13	740 ± 13	236 ± 5	1.7 ± 0.2	0.54 ± 0.08	0.72 ± 0.06
Sham-II	425 ± 17	732 ± 14	225 ± 7	1.8 ± 0.2	0.57 ± 0.07	0.73 ± 0.08
LVH-I	421 ± 12	1012 ± 1[a]	240 ± 7	2.4 ± 0.2[a]	0.56 ± 0.05	0.92 ± 0.13[a]
LVH-II	412 ± 19	1004 ± 15[a]	238 ± 8	2.2 ± 0.3[a]	0.55 ± 0.06	0.93 ± 0.14[a]
LVH-III	423 ± 15	987 ± 18[a]	229 ± 9	2.1 ± 0.2[a]	0.60 ± 0.08	0.90 ± 0.13[a]

Values are mean ± SD. Sham-I, papillary muscles isolated from sham-operated rats 10 weeks after operation; Sham-II, papillary muscles isolated from sham-operated rats 10 weeks after operation prior to addition of mibefradil; LVH-I, hypertrophied muscles isolated from 10 week aortic-banded rats; LVH-II, hypertrophied muscles isolated from 10 week aortic-banded rats prior to addition of mibefradil; LVH-III, hypertrophied muscles isolated from 10 week aortic-banded rats prior to addition of verapamil. 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, papillary muscle cross-sectional area. N = 8 in each group. [a]$P < 0.05$ vs. Sham-I and Sham-II.

Table II. Parameters of mechanical contractions and intracellular Ca^{2+} transients in papillary muscles isolated from sham-operated and aortic-banded rats at baseline

	DT (mN/mm^2)	TPT (ms)	RT_{50} (ms)	TPL (ms)	RL_{50} (ms)	Peak syst. $[Ca^{2+}]$ (μM)	Diastolic $[Ca^{2+}]_i$ (μM)
Sham-I	11.5 ± 2.1	92.2 ± 7.6	55.2 ± 3.4	37.7 ± 2.3	41.3 ± 3.4	0.64 ± 0.07	0.26 ± 0.03
Sham-II	11.2 ± 2.3	93.6 ± 8.2	56.2 ± 4.6	38.5 ± 1.8	41.5 ± 2.8	0.62 ± 0.05	0.25 ± 0.01
LVH-I	8.5 ± 1.2[a]	118.2 ± 10.2[a]	63.3 ± 6.1[a]	47.2 ± 2.8[a]	51.4 ± 3.3[a]	0.52 ± 0.02[a]	0.33 ± 0.05[a]
LVH-II	8.4 ± 0.9[a]	114.3 ± 9.7[a]	64.2 ± 4.7[a]	45.9 ± 3.1	48.1 ± 4.2	0.55 ± 0.06[a]	0.34 ± 0.04[a]
LVH-III	8.7 ± 1.0[a]	122.6 ± 9.5[a]	64.8 ± 5.2[a]	46.7 ± 2.9[a]	49.7 ± 3.5[a]	0.53 ± 0.04[a]	0.32 ± 0.03[a]

Values are mean ± SD. Sham-I, papillary muscles isolated from sham-operated rats 10 weeks after operation; Sham-II, papillary muscles isolated from sham-operated rats 10 weeks after operation prior to addition of mibefradil; LVH-I, hypertrophied muscles isolated from 10 week aortic-banded rats; LVH-II, hypertrophied muscles isolated from 10 week aortic-banded rats prior to addition of mibefradil; LVH-III, hypertrophied muscles isolated from 10 week aortic-banded rats prior to addition of verapamil. 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; Peak Syst. $[Ca^{2+}]_i$, peak systolic intracellular Ca^{2+}-concentration; Diastolic $[Ca^{2+}]_i$, diastolic intracellular Ca^{2+}-concentration. N = 8 in each group. [a]P < 0.05 vs. Sham-I and Sham-II.

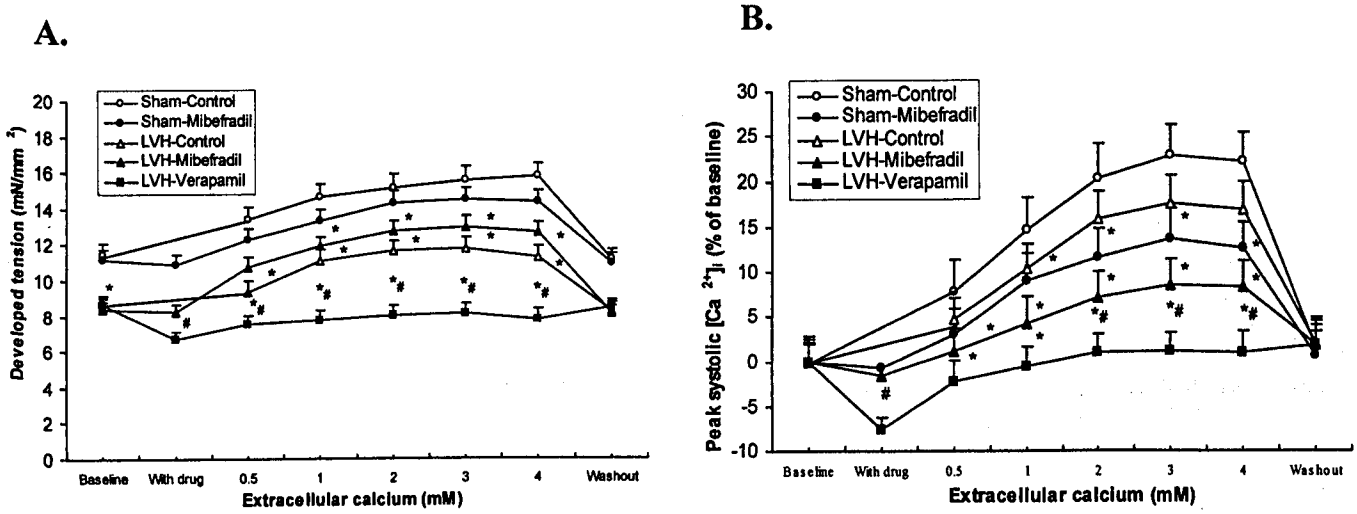

Figure 1. Changes in developed tension (A) and peak systolic intracellular Ca^{2+} (B, % changes of baseline) in response to extracellular Ca^{2+} stimulation in rat papillary muscles 10 weeks after operation. n = 8 in each group. Sham-Control, papillary muscles isolated from sham-operated rats without drug; Sham-Mibefradil, papillary muscles isolated from sham-operated rats with addition of mibefradil (2 μM); LVH-Control, hypertrophied muscles isolated from aortic-banded rats without drug; LVH-Mibefradil, hypertrophied muscles isolated from aortic-banded rats with addition of mibefradil (2 μM); LVH-Verapamil, hypertrophied muscles isolated from aortic-banded rats with addition of verapamil (2 μM). Washout, values measured after washout extracellular Ca^{2+}. *P < 0.05 vs. Sham-Control; #P < 0.05 LVH-Verapamil vs. LVH-Mibefradil.

trol group (Figs. 3 and 4). The reduction in amplitude of peak Ca^{2+} transients and isometric contraction to isoproterenol stimulation in hypertrophied papillary muscles with addition of verapamil is more pronounced than that of LVH-Mibefradil group.

The time courses of isometric contraction and relaxation showed no significant changes from increased $[Ca^{2+}]_o$ (data not shown), but exhibited shortening during isoproterenol stimulation in all muscle preparations isolated from sham-operated or aortic-banded rats (Fig. 5). The positive lusitropic effect to isoproterenol was attenuated in hypertrophied papillary muscles, and showed more pronounced attenuation with addition of verapamil (Fig. 5). No significant changes of time course in aequorin light signals were found during β-adrenergic receptor stimulation in all groups. Four hypertrophied papillary muscles (two from LVH-Control group and two from aortic-banded rats with addition of verapamil) exhibited prominent afterglimmers and corresponding aftercontractions at 10^{-5} and 10^{-4} M of isoproterenol stimulation (Fig. 4, A and C). After adding mibefradil (2 μM), afterglimmers and aftercontractions disappeared in hypertrophied muscle isolated from aortic-banded rats (Fig. 4A). Neither afterglimmers nor aftercontractions were observed during isoproterenol dose-response in hypertrophied muscles in the presence of mibefradil (Fig. 4B). The diastolic $[Ca^{2+}]_i$ was significantly increased in the aortic-banded group compared with sham-operated muscle preparations (Table II). The addition of mibefradil significantly decreased diastolic $[Ca^{2+}]_i$ (0.34 ± 0.04 μM in baseline vs. 0.28 ± 0.03 μM in the presence of 2 μM mibefradil; P < 0.05). However, no significant change of diastolic $[Ca^{2+}]_i$ was observed with addition of verapamil (0.32 ±

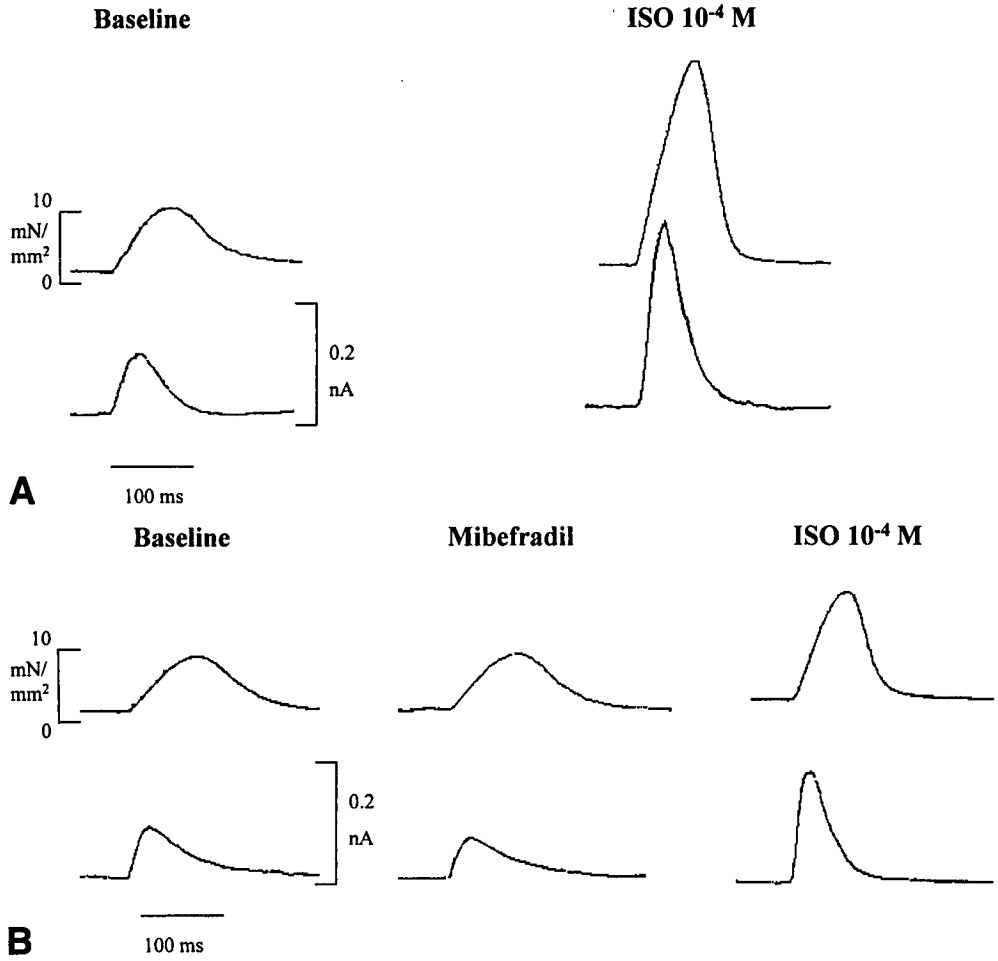

Figure 2. Aequorin light signal and isometric contraction in representative papillary muscles isolated from sham-operated rats (A) without mibefradil and (B) in the presence of mibefradil (2 μM). Mibefradil, in the presence of 2 μM of mibefradil; ISO, isoproterenol stimulation. Upper trace, isometric contraction; lower trace, aequorin light signal.

0.03 μM in the baseline vs. 0.31 ± 0.03 in the presence of 2 μM verapamil, P = NS).

Discussion

The major findings of the present study are that (i) mibefradil has a very weak negative inotropic effect on both normal and hypertrophied myocardium; that (ii) β-adrenergic stimulation in hypertrophied myocardium resulted in a reduction of myocardial contractility without a parallel decrease in $[Ca^{2+}]_i$ availability and showed more pronounced depression of isometric contraction in the presence of verapamil. This negative inotropic effect was partly restored by additional mibefradil without an accompanying increase in peak amplitude of $[Ca^{2+}]_i$; and that (iii) additional mibefradil rather than verapamil effectively antagonized the diastolic Ca^{2+} oscillations and corresponding aftercontractions, and further modified the cardiac disorders with impaired intracellular Ca^{2+} homeostasis.

Unlike most L-type Ca^{2+} channels, T-type Ca^{2+} channels activate and inactivate quickly, which is the reason for the transient openings of the T-type Ca^{2+} channels (9). The physiological role of the T-type Ca^{2+} channel is not clear. The largest T-type Ca^{2+} channels have been recorded in embryonic (19) and neonatal (20) ventricular myocytes, but are not present in normal adult feline ventricular myocytes (9, 14). The number of T-type Ca^{2+} channels increased when growth factors such as platelet-derived growth factor impact the heart (21), suggesting that T-type Ca^{2+} channels are associated with active cell growth and proliferation. Recently, Sen and Smith (12) reported with the whole-cell patch-clamp technique that the density of T-type Ca^{2+} channels in cardiac myocytes from cardiomyopathic hamster was significantly (more than 2-fold) higher than in normal cells, whereas the current density of L-type Ca^{2+} channels in normal cardiomyocytes was the same as that in cardiomyopathic myocytes. They suggest that intracellular Ca^{2+} overload in heart failure was induced by an increased influx of Ca^{2+} via more re-expressed T-type Ca^{2+} channels (12). Using a chronic feline model of left ventricular hypertrophy, Nuss and Houser (14) observed that T-type Ca^{2+} channels were re-expressed and the expression of these channels is stable throughout long-standing hypertrophy, but the density of L-type Ca^{2+} channels was reduced. The abnormal T-type Ca^{2+} channel properties might contribute to the pathogenesis of Ca^{2+} overload as a consequence of enhanced trans-sarcolemmal Ca^{2+} influx through this pathway. Our present study confirmed this hypothesis, i.e., reduction of L-type Ca^{2+} channels and abnormality of Ca^{2+} overload in hypertrophied myocardium might translate into

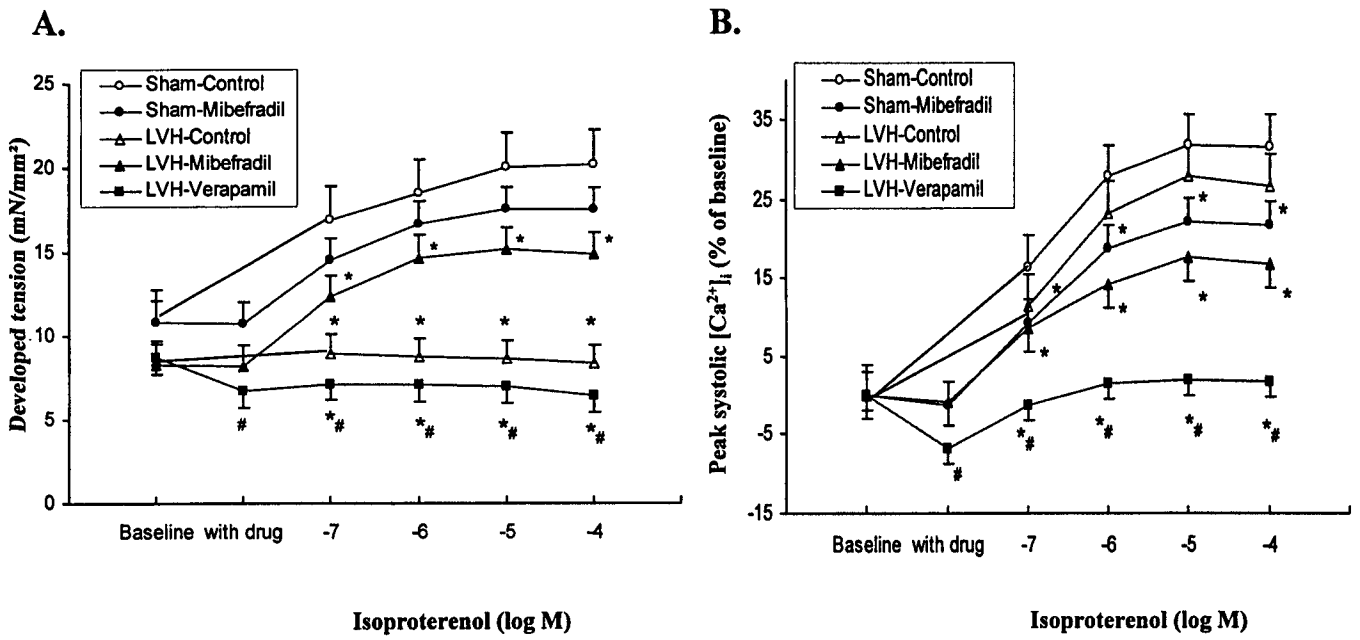

Figure 3. Line graphs summarizing the changes of developed tension and percentage of change of baseline peak systolic intracellular Ca^{2+} concentration to isoproterenol dose-response in papillary muscles isolated from sham-operated and aortic-banded rats 10 weeks after operation. Sham-Control, papillary muscles isolated from sham-operated rats without drug; Sham-Mibefradil, papillary muscles isolated from sham-operated rats with additional mibefradil (2 μM); LVH-Control, hypertrophied muscles isolated from aortic-banded rats without drug; LVH-Mibefradil, hypertrophied muscles isolated from aortic-banded rats with additional mibefradil (2 μM); LVH-Verapamil, hypertrophied muscles isolated from aortic-banded rats with additional verapamil (2 μM). $^{*}P < 0.05$ vs. Sham-Control; $^{\#}P < 0.05$ LVH-Verapamil vs. LVH-Mibefradil.

reduced Ca^{2+} release with a subsequent decrease of Ca^{2+} transient and isometric contractility.

Mibefradil, a new selective T-type Ca^{2+} channel antagonist (5, 6), has been used in treatment of hypertension (22, 23) and angina pectoris (23, 24) because of its unique properties. The difference with traditional L-type Ca^{2+} channel antagonists include (i) a slight heart-rate-lowering effect (25); (ii) the absence of reflex increases neurohormones and sympathetic activity with coronary and peripheral vasodilation (26); and (iii) the lack of a negative inotropic effect on cardiac contractility (27, 28). However, the direct influence of mibefradil on mechanical function and $Ca^{2+}{}_i$ handling in hypertrophied myocardium is unknown. The present study investigated a comparison of both T-type and L-type Ca^{2+} channel antagonists (mibefradil versus verapamil) on inotropy and $Ca^{2+}{}_i$ handling in hypertrophied rat myocardium after aortic banding. Additional mibefradil had no significant effects on isometric contraction in either sham or hypertrophied muscle preparations, which is consistent with previous findings (27, 28). The fact that mibefradil does not have negative inotropism at therapeutic concentrations might be due to less expression or absence of T-type channels in normal myocardium (11, 13, 14). The weak negative inotropy in hypertrophied myocardium with additional mibefradil treatment is presumably from either an increased Ca^{2+} responsiveness of the myofilaments or a modification of the diastolic Ca^{2+} homeostasis or, mutually related, both. Additional experiments are required to clarify this point. The depression of inotropic response to isoproterenol stimulation was partly restored in the presence of mibefradil, although there is no parallel increase of $[Ca^{2+}]_i$. However, the negative response to β-adrenergic stimulation was not restored with addition of verapamil in hypertrophied rat myocardium.

Increasing the concentration (10^{-5} and 10^{-4} *M*) of isoproterenol produced a prominent afterglimmer and corresponding aftercontraction due to spontaneous release of Ca^{2+} from the sarcoplasmic reticulum (SR). These findings are consistent with previous studies (15, 16) and they provide further evidence of SR dysfunction and subsequent induced Ca^{2+} overload and impaired heart contractility in hypertrophied myocardium. Reported data (29) showed that exposure to isoproterenol induced an increase in T-type Ca^{2+} current secondary to a rise in intracellular Ca^{2+} after augmentation of L-type Ca^{2+} current. The threshold for the opening of T-type Ca^{2+} channels is lower than that of L-type Ca^{2+} channels, and a light deviation from resting potential may generate a depolarizing "window" current via T-type Ca^{2+} channels that, in turn, could trigger spontaneous SR Ca^{2+} release (5). Mibefradil, a novel T-type Ca^{2+} channel antagonist, could block a large re-expression of T-type Ca^{2+} channels in hypertrophied myocardium, modify Ca^{2+} overload, and improve inotropic response to β-adrenergic stimulation.

It has been demonstrated that there is a reduction of the SR Ca^{2+}-ATPase in hypertrophied rat hearts (30), subsequently leading to a decrease in reuptake of Ca^{2+} by the SR. Previous experiments (15–17) demonstrated the descending phase of the calcium transient predominantly reflects resequestration of calcium by the SR. The afterglimmers of

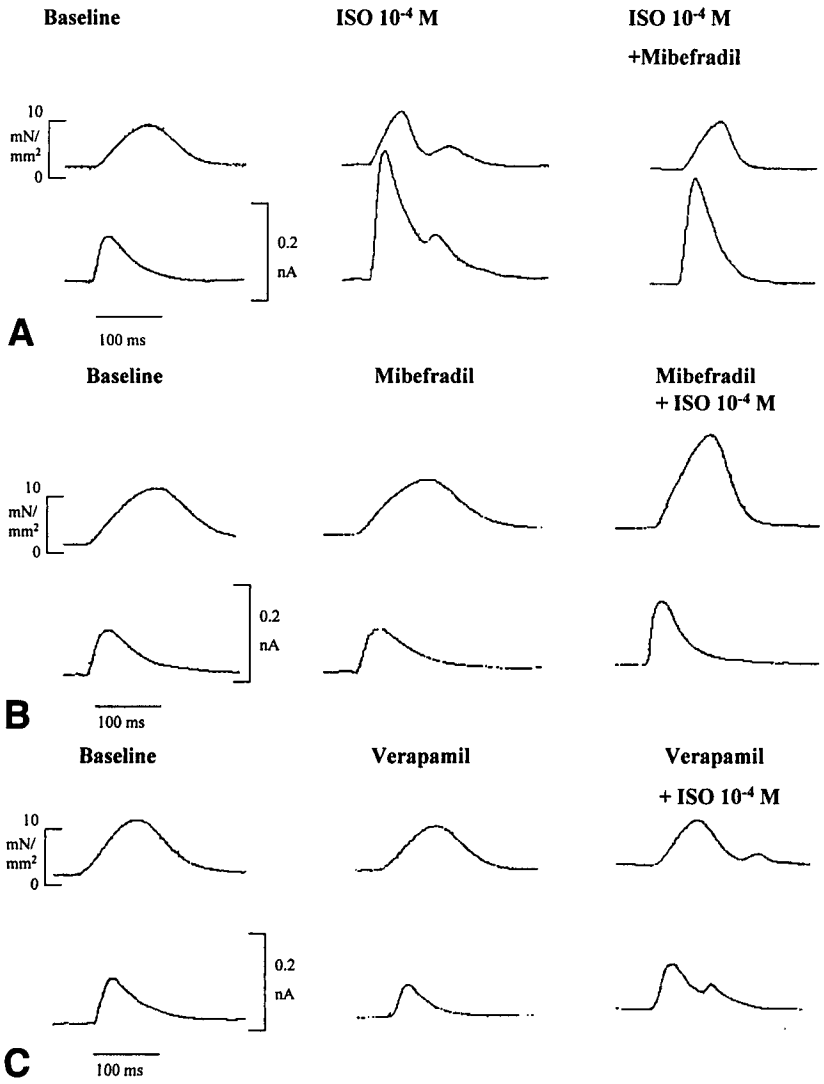

Figure 4. Aequorin light signal and isometric contraction in representative hypertrophied papillary muscles isolated from 10 week aortic-banded rats (A) without drug, (B) in the presence of mibefradil (2 μM), and (C) in the presence of verapamil (2 μM). Mibefradil, in the presence of 2 μM of mibefradil; Verapamil, in the presence of 2 μM of verapamil; ISO, isoproterenol stimulation. Results show isometric contraction (upper trace) and aequorin light signal (lower trace).

Ca^{2+} transients were observed in 4 out of 16 hypertrophied rat papillary muscles during isoproterenol stimulation in the present study, which provided further evidence of impaired SR function in cardiac hypertrophy. Afterglimmers of Ca^{2+} transients were observed to be associated with high doses of β-agonist isoproterenol in four hypertrophied muscles (two from LVH-Control group, and two from LVH-Verapamil group), which corresponded to appearances of mechanical aftercontractions. This spontaneous Ca^{2+} release from the SR during diastole is the basic character of Ca^{2+} overload, which induces contractile and electrophysiological dysfunction (31). In Ca^{2+} overload states, Ca^{2+} was gradually transferred within the SR from the uptake site to release site, causing efflux of some Ca^{2+} from the release site into the cytosol. Subsequently, a rise in intracellular Ca^{2+} can induce an afterglimmer and aftercontraction by interacting with the myofilament. Intracellular Ca^{2+} overload in hypertrophied myocardium might relate to a considerable increase of the T-type Ca^{2+} channel current density by enhanced transsarcolemmal Ca^{2+} influx through the increased T-type channels, in addition to reduction of Ca^{2+} uptake by SR dysfunction. Mibefradil selectively blocks the T-type Ca^{2+} channel, modifies impaired SR function, and blocks the spontaneous Ca^{2+} release from the SR during diastole, i.e., reduced diastolic intracellular Ca^{2+} concentration and antagonized afterglimmers of $Ca^{2+}{}_i$ transients in the present study in hypertrophied rat myocardium. Verapamil, a L-type Ca^{2+} channel antagonist, has a strong negative inotropic effect in hypertrophied rat papillary muscles induced by aortic banding. The negative inotropic response to isoproterenol stimulation in hypertrophied rat myocardium was not restored with the addition of verapamil. The present study suggests that verapamil does not affect impaired $Ca^{2+}{}_i$ homeostasis and that it deteriorates abnormal intracellular Ca^{2+} handling in hypertrophied myocardium.

Our results indicate that mibefradil, a novel T-type Ca^{2+} channel antagonist, is quite effective in reducing diastolic Ca^{2+} oscillations, modifying abnormal Ca^{2+} handling, and improving inotropic effects of β-adrenergic stimulation in hypertrophied rat myocardium. Thus, mibefradil could be beneficial and become a valuable clinical therapeutic tool in the hypertrophied heart.

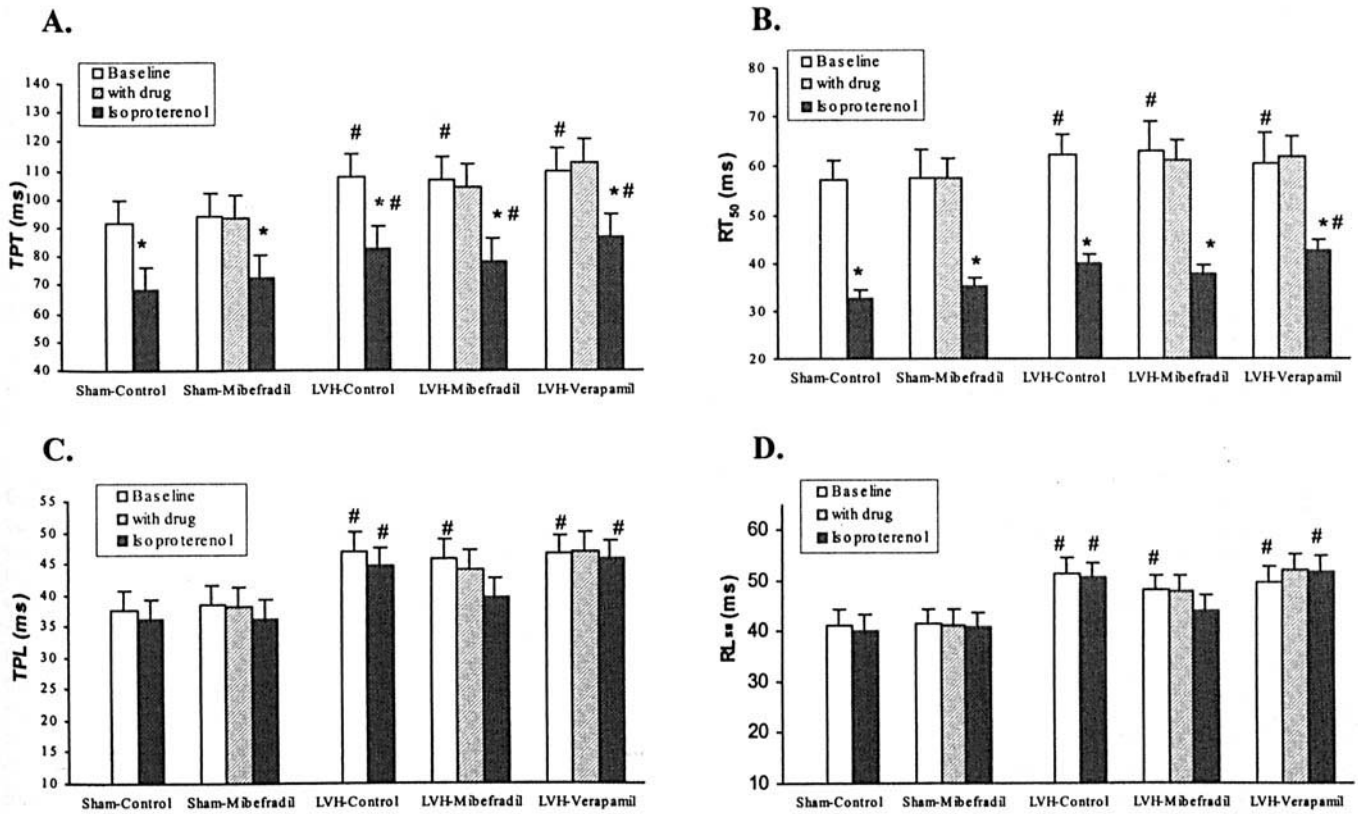

Figure 5. Bar graphs showing time courses of isometric contraction and Ca^{2+} transients to isoproterenol dose-response in all animals 10 weeks after operation. Sham-Control, papillary muscles isolated from sham-operated rats without drug; Sham-Mibefradil, papillary muscles isolated from sham-operated rats with addition of mibefradil (2 μM); LVH-Control, hypertrophied muscles isolated from aortic-banded rats without drug; LVH-Mibefradil, hypertrophied muscles isolated from aortic-banded rats with addition of mibefradil (2 μM); LVH-Verapamil, hypertrophied muscles isolated from aortic-banded rats with addition of verapamil (2 μM). (A) Time to peak tension (TPT); (B) time from peak tension to 50% relaxation (RT_{50}); (C) time to peak light (TPL); (D) time from peak light to 50% decline (RL_{50}). *P <0.05 values at 10^{-4} M of isoproterenol vs. Baseline in each group; #P < 0.05 vs. Sham-Control at Baseline or at 10^{-4} M of isoproterenol.

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