

Differential Effects of Mibefradil, Verapamil, and Amlodipine on Myocardial Function and Intracellular Ca^{2+} Handling in Rats with Chronic Myocardial Infarction

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Accepted for publication August 12, 1999 This paper is available online at <http://www.jpvet.org>

ABSTRACT

Mibefradil is a selective T-type Ca^{2+} channel blocker that exerts a potent vasodilating but weak inotropic action. The present study compared mibefradil with traditional L-type Ca^{2+} channel blockers in regard to the effects of chronic oral administration on hemodynamics, contractility, and intracellular Ca^{2+} handling in failing myocardium from postinfarction rats. Male Wistar rats with ligation-induced myocardial infarction were assigned to placebo or treatment with mibefradil (10 mg/kg/day), verapamil (8 mg/kg/day), or amlodipine (4 mg/kg/day) by oral gavage starting 7 days before the induction of myocardial infarction. Six weeks after myocardial infarction, hemodynamic measurements were performed in conscious animals. In addition, isometric force and free $[\text{Ca}^{2+}]_i$ were determined in isolated left ventricular papillary muscles. Placebo-treated rats exhibited a decreased mean atrial pressure, an increased left ventricular end-diastolic pressure, and a reduced rate of pres-

sure rise compared with sham-operated animals. Mibefradil treatment significantly improved all of these parameters, whereas both amlodipine and verapamil exerted only minor effects. β -Adrenergic stimulation with isoproterenol (ISO) enhanced contractility and Ca^{2+} availability in papillary muscles from sham-operated rats, whereas the ISO-induced inotropic effect in muscles from placebo-treated rats was severely blunted. Chronic mibefradil treatment significantly improved the inotropic response to ISO stimulation, although the Ca^{2+}_i availability appeared to be less than in muscles from placebo-treated animals. In contrast, both verapamil and amlodipine did not restore the inotropic and Ca^{2+}_i modulating effect of ISO in remodeled myocardium. Thus, T-type Ca^{2+} current appears to be of pathophysiological relevance in posts ischemic reperfused myocardium.

Calcium channel blockers (CCBs) have been extensively used in the treatment of cardiovascular disease (Braunwald, 1982; Held et al., 1989). However, recent clinical studies raised concerns about their potential negative effects on morbidity and mortality in patients with coronary artery disease and hypertension (Yusuf, 1995). In particular, the use of CCBs in chronic myocardial infarction (MI) did not improve or even deteriorate the odds of adverse clinical outcomes (Held et al., 1989; Diltiazem Postinfarction Trial Group, 1989). The conventional CCBs are subdivided into three major chemical classes (dihydropyridines, phenylalkylamines, and benzothiazepines) but share a common mechanism of action, i.e., selective inhibition of the transmembrane flux of calcium into the cell via L-type (long-lasting, high voltage-activated) Ca^{2+} channels (Opie et al., 1987). The resulting vasodilation and negative inotropy potentially induce a reflex increase in sympathetic tone that may negatively affect prognosis (Noll et al., 1988).

Mibefradil is a member from a new chemical class of CCBs (benzimidazolyl-substituted tetraline derivative) that is ~30 to 100 times more potent in blocking T-type (transient, low voltage-activated) than L-type Ca^{2+} channels in vascular smooth muscle (Mishra and Hermsmeyer, 1994). Conversely, CCBs of the dihydropyridine, phenylalkylamine, and benzothiazepine classes have no ability to block T-type calcium channels at therapeutic concentrations (Triggle, 1991). T-type Ca^{2+} channels are mainly present in smooth muscle and sinus node cells but also are found in rapidly proliferating cells as well as hypertrophied cardiac myocytes (Hagiwara et al., 1988; Nuss and Houser, 1993; Schmitt et al., 1995; Katz, 1996).

The in vivo effects of mibefradil include potent peripheral vasodilation, coronary vasodilation, and a decrease in heart rate (Luescher et al., 1997). At therapeutic concentrations, the drug exerts no negative inotropic action and does not stimulate the sympatho-adrenergic reflex activity (Schmitt et al., 1992; Su et al., 1994). Inhibition of vascular smooth muscle and mesangial cell proliferation by mibefradil posi-

Received for publication May 12, 1999.

ABBREVIATIONS: CCB, calcium channel blocker; MI, myocardial infarction; MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure; DT, developed tension; ISO, isoproterenol.

tively affects vascular remodeling in hypertension (Hermsmeyer and Miyagawa, 1996). Similarly, mibefradil has been demonstrated to induce beneficial effects on the cardiac remodeling process after acute myocardial infarction in rats (Mulder et al., 1997; Sandmann et al., 1998).

Contractile dysfunction in failing myocardium has been attributed to alterations of the intracellular Ca²⁺ homeostasis (Morgan et al., 1990). T-type Ca²⁺ current is negligible in normal adult ventricular but is re-expressed by the reactivated fetal gene program in remodeled and hypertrophied ventricular myocytes (Nuss and Houser, 1993; Qin et al., 1995). The pathophysiological relevance of this phenomenon is unknown but might be related to arrhythmias and mechanical impairment in postinfarction myocardium. Therefore, the purpose of this study was to investigate the hemodynamic, inotropic, and [Ca²⁺]_i-modulating effects of chronic mibefradil treatment in the infarcted rat heart and to perform a comparison with L-type CCBs exhibiting a prominent cardiac (verapamil) or vascular (amlodipine) site of action at therapeutic concentrations.

Materials and Methods

The experiments were performed in male Wistar rats (Charles River Viga, Sulzfeld, Germany) with an initial body weight of 250 to 300 g. The animals were housed individually under climate-controlled conditions with a 12-h light/dark cycle and provided with standardized rat chow and tap water ad libitum. The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication 85-23, revised 1985).

Animal Model of MI. MI was induced by left anterior descending coronary artery ligation according to a previously described technique (Pfeffer et al., 1985). Endotracheal intubation was performed and followed by artificial ventilation during anesthesia with ether. Subsequently, anesthesia was maintained with i.v. methohexital sodium given via the tail vein. The chest was opened by a left-sided sternal incision. A rib-spreading chest retractor was inserted and the left anterior descending coronary artery was ligated by atraumatic suture with 6.0 sterile silk. The successful ligation of the coronary artery was verified by surface ECG recording and visual change of color in the infarcted area. The thoracic cavity was closed by interrupted sutures. The sham-operated rats underwent an identical surgical procedure without coronary ligation.

Drug Randomization. Previous studies demonstrate that the protective effects of L-type Ca²⁺ channel blockers on ischemic, reperfused, and postischemic myocardium become most evident if the drug regimen starts before the induction of ischemia (Nayler et al., 1978; Klein et al., 1984; Lo et al., 1985). Therefore, the study protocol used a similar approach to maximize beneficial effects and allow for comparisons between a reasonable number of animals in each treatment group. The rats were treated with mibefradil (10 mg/kg/day; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany), verapamil (8 mg/kg/day; Sigma-Aldrich GmbH, Deisenhofen, Germany), amlodipine (4 mg/kg/day; Pfizer GmbH, Karlsruhe, Germany), or placebo by oral gavage once daily starting 7 days before induction of MI. In a previous set of experiments, these dosages had been tested and shown to not significantly alter the mean arterial blood pressure in infarcted or sham-operated animals. This regimen was continued in the surviving animals until sacrifice at 6 weeks post-MI. A total of seven or eight rats was operated on in each group. The peri-infarction mortality (1 or 2 rats in each group) was similar in all groups so that the study cohort comprised a total of 30 surviving rats with 6 animals in each of the following groups: untreated sham-operated rats (sham group), placebo-treated rats with coronary ligation (placebo-MI group), mibefradil-treated rats with coronary ligation (mi-

befradil-MI group), verapamil-treated rats with coronary ligation (verapamil-MI group), and amlodipine-treated rats with coronary ligation (amlodipine-MI group).

Hemodynamic Measurements. Six weeks after MI or sham operation, chronic arterial and venous catheters, as well as a catheter in the left ventricle, were implanted to measure hemodynamic data. Anesthesia was initiated with diethylether and continued by i.v. methohexital-sodium (10 mg/kg). Femoral arterial and venous catheters were chronically implanted with a procedure described previously (Unger et al., 1984). In short, polypropylene tubes (Portex, London, UK) were inserted into the right femoral artery and vein and exteriorized at the nape of the neck. The right carotid artery was cannulated with a specially constructed pig-tail catheter (Stauss et al., 1994) that was retrogradely passed across the aortic valve and advanced into the left ventricle. The distal portion of the catheter was then tunneled under the skin and anchored at the posterior neck region. The hemodynamic studies were performed 24 h later in conscious animals with conventional pressure transducers (DTX/Plus; Spectramed Inc, Oxnard, CA) and processors (Gould Inc., Cleveland, OH). Mean arterial blood pressure (MAP), heart rate, left ventricular end-diastolic pressure (LVEDP), and maximum positive change of left ventricular pressure (dp/dtmax) were documented on a pen recorder (Gould Series 2000; Gould Inc.) and processed with a computer-based analyzing system as described previously (Stauss et al., 1990).

Isometric Muscle Performance. Immediately after completion of the hemodynamic measurements, the rats were sacrificed during deep anaesthesia with ether. The heart was rapidly excised and placed in a dissecting chamber containing a modified Krebs-Henseleit solution of the following composition: 120 mM NaCl, 5.9 mM KCl, 5.5 mM dextrose, 2.5 mM NaHCO₃, 12 mM NaH₂PO₄, 1.2 mM MgCl₂, 1.0 mM CaCl₂, pH 7.4, bubbled with carbogen (a mixture of 95% O₂ and 5% CO₂) at room temperature. The noninfarcted left ventricular papillary muscle was carefully dissected and then fixed to a muscle holder with a spring clip. The tendinous end of the muscle was vertically connected to a strain-gauge tension transducer (Type 372; Hugo Sachs Elektronik GmbH, Freiburg, Germany) with a silk thread. The muscle was then mounted in a 50-ml tissue bath containing modified Krebs-Henseleit solution maintained at 30°C and continuously bubbled with carbogen. The isometric contraction of the papillary muscle was elicited by a punctate platinum electrode with square-wave pulses of 5-ms duration at 0.33 Hz. The voltage was set to 10% above threshold level. After a 30-min equilibration period, the muscle was carefully stretched to the length at which maximal tension occurred (L_{max}). The following isometric contraction parameters were recorded from each muscle at this maximal length: developed tension (DT, tension produced by the stimulated muscle), time to peak tension (time from the beginning of the contraction to peak tension), and time to 50% relaxation (time from peak tension to 50% of relaxation). Subsequently, the loading procedure for aequorin was performed (see below). At the end of the experiment, the muscle was blotted and weighed. The cross-sectional area was determined from muscle weight and length by assuming a uniform cross-section and a specific gravity of 1.05. After removal of the papillary muscle for study, the weights of the right and left ventricle, including the septum, were normalized by body weight and used as indices of hypertrophy.

Aequorin Light Signal Measurement. Aequorin (courtesy of Dr. John Blinks, Friday Harbor Laboratory, Friday Harbor, WA) was loaded into the nonstimulated muscle preparation by macroinjection technique (Meissner et al., 1996). Briefly, the preparation was raised from the organ bath and 1 to 2 μM aequorin solution (1 mg/ml) was injected under the epimysium at the base of the muscle with a short-shank low-resistance glass micropipette. After an equilibration period of 90 to 120 min, the stimulation was restarted at 0.33 Hz. The aequorin light signal was detected with photomultiplier tube (PM28B; Thorn EMI Electron Tubes, Rockaway, NJ) and converted into a voltage signal. Analog signals from the isometric force trans-

ducer and electronic photometer were recorded with a chart-strip recorder (model 56-1X 40-006158; Gould Inc.) and stored on videotape (Model HR-J400U; JVC Company of America, Elmwood Park, NJ). To improve the signal-to-noise ratio, 8 to 64 steady-state light signals and isometric twitches were averaged with a digital oscilloscope (model 5460113, 100 MHz; Hewlett-Packard GmbH, Böblingen, Germany) for quantitative measurements.

Parameters derived from the light signals included the amplitude of the light transient, time to peak light, and time from peak to 50% fall in peak light (RL_{50}). The free $[Ca^{2+}]_i$ was estimated by normalizing the recorded light signal during isometric twitches (L) by the maximal amount of light emitted after lysis of the muscle membranes (Lmax) at the end of experiment with a 5% solution of the detergent Triton X-100 in phosphate-free physiological salt solution containing 50 mM Ca^{2+} . The normalized light signal was then converted to $[Ca^{2+}]_i$ with an in vitro calibration curve as previously reported (Meissner et al., 1996).

Isoproterenol (ISO) Dose-Response Determinations. After baseline parameters were obtained, ISO (10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} M; Sigma-Aldrich GmbH, Deisenhofen, Germany) was added cumulatively to determine the inotropic response to β -adrenergic stimulation. Light signals and isometric contractions were measured 10 min after each dose of ISO.

Statistical Analysis. All values are given as means \pm S.E. Data were evaluated by one-way ANOVA with repeated measures. Differences between individual groups were compared by using Student's *t* test and considered significant at $p < .05$.

Results

Morphology and Hemodynamics. Six weeks after infarction, the left ventricular weight and left ventricular weight-to-body weight ratio in placebo-treated rats were significantly increased compared with sham-operated animals (Table 1). Chronic treatment with CCBs decreased left ventricular hypertrophy to a similar degree in all groups. Animals in the placebo-MI group exhibited a smaller rate of pressure rise (dP/dtmax), a lower MAP, and a LVEDP than sham-operated rats (Table 2). Chronic treatment with mibefradil (mibefradil-MI group) significantly improved dP/dtmax and MAP in infarcted rats. LVEDP was lowered but remained significantly higher than in the sham-operated group. In contrast, chronic treatment with verapamil (verapamil-MI group) had no beneficial effect on the hemodynamic parameters. Chronic treatment with amlodipine (amlodipine-MI group) induced only moderate improvements of dP/dtmax, MAP, and LVEDP, which were distinctly less in magnitude than those in the mibefradil-MI group.

Isometric Force Measurements. At baseline, the isolated, noninfarcted papillary muscles in the placebo-MI

TABLE 2

Hemodynamic data in sham-operated and MI rats
Values are means \pm S.E.

	HR	MAP	LVEDP	dP/dtmax
	<i>bpm</i>	<i>mm Hg</i>	<i>mm Hg</i>	<i>mm Hg/s</i>
Sham	361 \pm 5.6	111 \pm 2.1	10.2 \pm 1.1	8.6 \pm 0.5
Placebo-MI	373 \pm 6.2	82 \pm 2.0 ^a	21.1 \pm 2.3 ^a	4.9 \pm 0.5 ^a
Mibefradil-MI	369 \pm 5.4	102 \pm 2.4 ^b	14.1 \pm 2.7 ^{c,b}	7.1 \pm 0.4 ^{c,b}
Verapamil-MI	343 \pm 4.6	81 \pm 2.2 ^a	20.0 \pm 2.8 ^{a,d}	5.3 \pm 0.6 ^{a,e}
Amlodipine-MI	372 \pm 5.1	96 \pm 2.3 ^{c,f}	18.1 \pm 2.4 ^{a,e}	5.9 \pm 0.5 ^{a,e}

sham, sham-operated rats; placebo-MI, infarcted rats at 6 weeks post-MI with placebo treatment; mibefradil-MI, infarcted rats at 6 weeks post-MI with mibefradil treatment; verapamil-MI, infarcted rats at 6 weeks post-MI with verapamil treatment; amlodipine-MI, infarcted rats at 6 weeks post-MI with amlodipine treatment ($n = 6$ in each group).

^a $p < .01$ versus sham group.

^b $p < .01$ versus placebo-MI group.

^c $p < .05$.

^d $p < .01$ versus mibefradil-MI group.

^e $p < .05$.

^f $p < .05$.

group exhibited a slightly reduced DT and a prolonged time course of the isometric twitch compared with papillary muscles from sham-operated animals (Table 3). In the mibefradil-MI group, DT appeared to be almost completely preserved, whereas it was significantly decreased in both the verapamil-MI and amlodipine-MI group. The time course of contraction was prolonged to a similar extent in all drug-treated groups.

β -Adrenergic stimulation with cumulative concentrations of ISO induced a pronounced increase in DT in normal papillary muscles from sham-operated rats (Figs. 1A and 2A). In contrast, no positive inotropic effect was observed during ISO application in the placebo-MI group (Fig. 2B). Chronic treatment with mibefradil partly restored the inotropic response to ISO in the mibefradil-MI group, whereas in both the verapamil-MI and amlodipine-MI group ISO stimulation failed to augment DT (Fig. 3, A–C).

Systolic $[Ca^{2+}]_i$ Measurements. At baseline, the peak systolic $[Ca^{2+}]_i$ in isolated noninfarcted papillary muscles of the placebo-MI group and in papillary muscles of the sham-operated group were equivalent (Table 3). In the mibefradil-MI group, systolic $[Ca^{2+}]_i$ was found at a similar level, whereas it was significantly reduced in both the verapamil-MI and amlodipine-MI group. The time course of the Ca^{2+} transient exhibited a significant prolongation in the placebo-MI, verapamil-MI, and amlodipine-MI groups but only a minor change in the mibefradil-MI group (Fig. 4).

Cumulative ISO-stimulation increased peak systolic $[Ca^{2+}]_i$ in parallel to DT in papillary muscles from sham-

TABLE 1

General characteristics of sham-operated and MI rats
Values are means \pm S.E.

	BW	LVW	RVW	LVW/BW	RVW/BW	CSA
		<i>mg</i>			<i>mg/g</i>	
Sham	423 \pm 24.7	749 \pm 54.5	273 \pm 41.1	1.78 \pm 0.07	0.65 \pm 0.12	0.71 \pm 0.07
Placebo-MI	409 \pm 16.5	1004 \pm 53.2 ^a	278 \pm 58.5	2.55 \pm 0.15 ^a	0.69 \pm 0.16	0.92 \pm 0.09 ^a
Mibefradil-MI	420 \pm 25.7	792 \pm 38.6	281 \pm 39.9	1.89 \pm 0.13	0.67 \pm 0.11	0.69 \pm 0.08
Verapamil-MI	408 \pm 13.1	810 \pm 56.5	279 \pm 31.7	1.97 \pm 0.12	0.72 \pm 0.18	0.75 \pm 0.12
Amlodipine-MI	418 \pm 32.4	791 \pm 30.7	267 \pm 30.4	1.90 \pm 0.15	0.64 \pm 0.03	0.74 \pm 0.10

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; sham, papillary muscles from sham-operated rats; placebo-MI, papillary muscles from MI rats with placebo-treatment at 6 weeks post-MI; mibefradil-MI, papillary muscles from MI rats with mibefradil treatment at 6 weeks post-MI; verapamil-MI, papillary muscles from MI rats with verapamil treatment at 6 weeks post-MI; amlodipine-MI, papillary muscles from MI rats with amlodipine treatment at 6 weeks post-MI.

^a $p < .05$ versus sham, mibefradil-MI, verapamil-MI, and amlodipine-MI group.

TABLE 3

Parameters of mechanical contractility and free intracellular Ca²⁺ concentration in isolated papillary muscles from sham-operated and MI rats with placebo or drug administration

Values are means ± S.E.

	DT	TPT	RT ₅₀	TPL	RL ₅₀	Systolic [Ca ²⁺] _i	Diastolic [Ca ²⁺] _i
	mN/mm ²		ms		μM		
Sham	12.8 ± 0.15	87.3 ± 8.4	55.3 ± 4.5	38.2 ± 2.8	44.8 ± 4.1	0.61 ± 0.07	0.28 ± 0.03
Placebo-MI	11.7 ± 0.19	103.4 ± 9.5*	59.5 ± 5.2*	45.7 ± 4.2**	51.2 ± 4.4*#	0.60 ± 0.09	0.35 ± 0.05*#
Mibefradil-MI	12.3 ± 0.16	101.5 ± 8.7*	57.2 ± 4.8	41.3 ± 2.7*	45.3 ± 3.6	0.57 ± 0.04	0.29 ± 0.05
Verapamil-MI	7.3 ± 0.14**	106.4 ± 9.8*	61.5 ± 6.1*	43.2 ± 3.1*	50.3 ± 2.5*#	0.51 ± 0.02*	0.34 ± 0.02*#
Amlodipine-MI	8.4 ± 0.20**	105.2 ± 9.6*	60.1 ± 5.2*	42.6 ± 2.3*	49.6 ± 2.8*#	0.52 ± 0.03*	0.33 ± 0.02*#

TPT, time to peak tension; RT₅₀, time from peak tension to 50% relaxation; TPL, time to peak light signal; RL₅₀, time from peak light to 50% decline; Systolic [Ca²⁺]_i, peak systolic free [Ca²⁺]_i concentration; Diastolic [Ca²⁺]_i, diastolic free [Ca²⁺]_i concentration; sham, papillary muscles from sham-operated rats; placebo-MI, papillary muscles from MI rats at 6 weeks post-MI; mibefradil-MI, papillary muscles from MI rats with mibefradil treatment at 6 weeks post-MI; verapamil-MI, papillary muscles from MI rats with verapamil treatment at 6 weeks post-MI; amlodipine-MI, papillary muscles from MI rats with amlodipine treatment at 6 weeks post-MI.

* *p* < .05.

** *p* < .01 versus sham group.

p < .05 versus mibefradil-MI group.

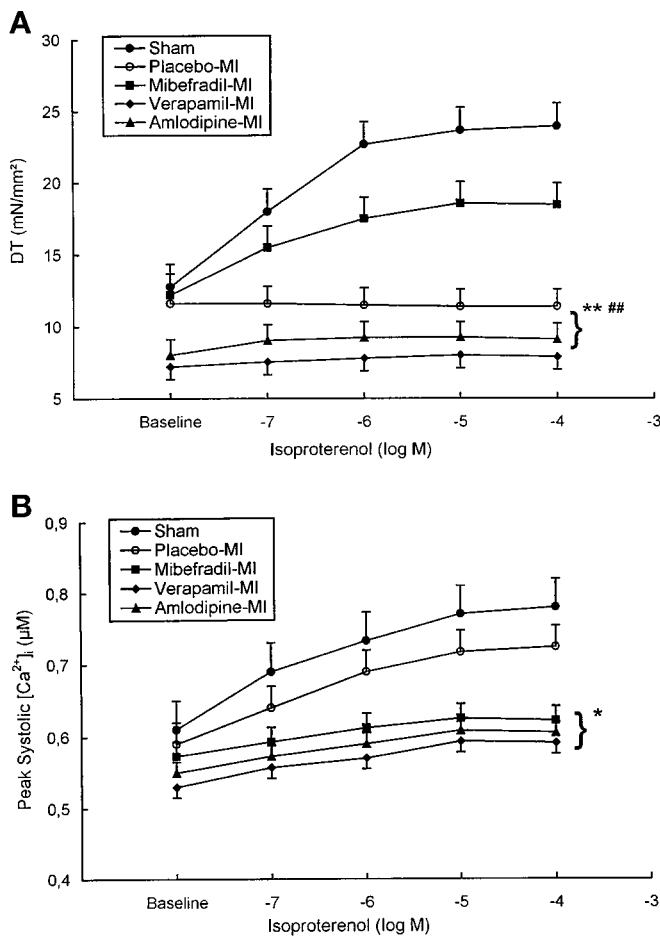


Fig. 1. DT (A) and peak systolic [Ca²⁺]_i (B) in response to ISO stimulation in papillary muscles from sham-operated and MI rats at 6 weeks post-MI. Sham, sham-operated group; placebo-MI, MI group with placebo treatment; mibefradil-MI, MI group with mibefradil treatment; verapamil-MI, MI group with verapamil treatment (*n* = 6 in each group). **p* < .05, ***p* < .01 versus sham-operated group at ISO (10⁻⁶, 10⁻⁵, 10⁻⁴ M); ##*p* < .01 versus mibefradil-MI group at ISO (10⁻⁶, 10⁻⁵, and 10⁻⁴ M).

operated rats (Fig. 1B). In the placebo-MI group, this ISO effect was well preserved despite the fact that no inotropic response occurred. A reverse phenomenon was observed in the mibefradil-MI group, where ISO stimulation induced a small increase in systolic [Ca²⁺]_i but a pronounced inotropic effect. In both the verapamil-MI and amlodipine-MI group,

β-adrenergic stimulation with ISO also produced only a minor increase of the peak systolic [Ca²⁺]_i (Fig. 3, A–C).

Diastolic [Ca²⁺]_i and Ca²⁺ Oscillations. At baseline, the diastolic [Ca²⁺]_i was significantly elevated in noninfarcted papillary muscles of the placebo-MI, verapamil-MI, and amlodipine-MI groups compared with normal muscles in the sham-operated group (Table 3). In contrast, diastolic [Ca²⁺]_i was not increased in the mibefradil-MI group. β-Adrenergic stimulation with ISO at a final concentration of 10⁻⁴ M consistently elicited diastolic Ca²⁺ oscillations in all papillary muscles of the placebo-MI, verapamil-MI, and amlodipine-MI group, whereas no such phenomena could be provoked in the mibefradil-MI and sham-operated group (Figs. 1, A–B and 3, A–C).

Discussion

Postinfarction myocardial remodeling is a complex transformation of left ventricular morphology and function involving both infarcted and noninfarcted areas. This process includes dilatation and thinning of the infarcted left ventricular wall segments as well as a compensatory reactive hypertrophy of the remaining viable myocardium (Pfeffer and Braunwald, 1990). Experimental studies suggest that L-type Ca²⁺ channel blockers may provide protective effects in the ischemic and reperfused heart by way of a modulation of the myocardial Ca²⁺ homeostasis (Naylor et al., 1980; Amende et al., 1992). Moreover, intracellular Ca²⁺ homeostasis seems to play a permissive or potentiating role in activating several second messenger systems involved in the regulation of myocardial growth and hypertrophy (Sadoshima and Izumo, 1993). In contrast, the results of clinical trials with L-type CCBs in patients with myocardial infarction have been negative and do not support a therapeutic concept based upon intracellular Ca²⁺ modulation.

The present study demonstrates that chronic treatment with verapamil, amlodipine, and mibefradil reduced compensatory left ventricular hypertrophy to a similar degree in the postinfarction rat model. This finding may reflect a beneficial effect on the myocardial remodeling process resulting from an initial reduction of infarct size (Weishaar and Bing, 1980; Sandmann et al., 1998). Surprisingly, this common morphological feature in the drug-treated animals was not associated with equally uniform changes of the hemodynamic situation. Chronic treatment with verapamil and amlodipine had no or only minor effects on LVEDP, MAP, and dp/dtmax

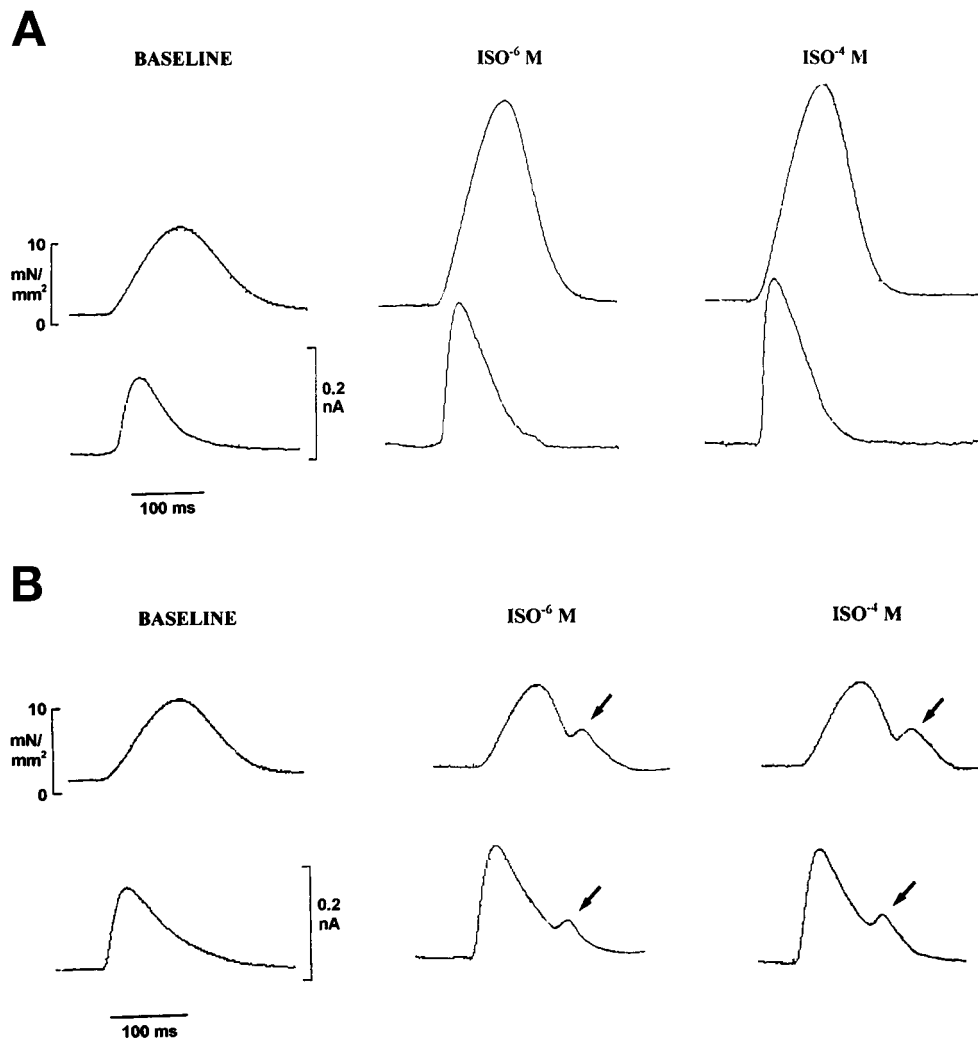


Fig. 2. Aequorin light signal and isometric contraction from representative rat papillary muscles of the sham-operated (A) and placebo-treated MI group (B) during ISO stimulation. Upper trace, isometric contraction; lower trace, light signal. Diastolic Ca^{2+} oscillations and mechanical aftercontractions are marked by arrows.

compared with those of placebo-treated animals. In contrast, mibefradil significantly reduced preload (LVEDP), improved contractility (dP/dt_{max}), and increased MAP in chronically infarcted rats, confirming the results of a previous study (Sandmann et al., 1998). Thus, T-type Ca^{2+} channel blockade with mibefradil induces an improvement of myocardial function that cannot solely be attributed to its effects on left ventricular hypertrophy but might be related to changes of the intracellular Ca^{2+} homeostasis. Myocardium from rats with postinfarction heart failure has been shown to exhibit a severely blunted inotropic response to β -adrenergic stimulation despite a large increase in the amplitude of the Ca^{2+} transient (Litwin and Morgan, 1992). The present study shows that chronic treatment with verapamil or amlodipine resulted in a further deterioration of isometric force as well as a reduction of the systolic Ca^{2+} availability during ISO stimulation. In contrast, chronic treatment with mibefradil partially restored the β -adrenergic, inotropic responsiveness in remodeled myocardium from postinfarction rats although the systolic $[\text{Ca}^{2+}]_i$ remained depressed. It is tempting to speculate that this phenomenon is due to either an increased Ca^{2+} responsiveness of the myofilaments or a modification of the diastolic Ca^{2+} homeostasis or, mutually related, both. Elevation of $[\text{Ca}^{2+}]_i$ induces changes in protein synthesis and degradation as well as activation of phosphorylating and

proteolytic enzymes that affect the contractile proteins (Kusuoka et al., 1990; Sadoshima et al., 1995). Lowering of diastolic $[\text{Ca}^{2+}]_i$ by chronic mibefradil treatment may have ameliorated these perturbations resulting in a preserved myofibrillar Ca^{2+} responsiveness.

Abnormal intracellular Ca^{2+} handling has been suggested as a major source of contractile dysfunction in failing myocardium (Morgan et al., 1990). In particular, elevation of the diastolic $[\text{Ca}^{2+}]_i$ may generate temporal and spatial inhomogeneities of $[\text{Ca}^{2+}]_i$ which, in turn, increase diastolic tone, reduce systolic force generation, and trigger arrhythmias (Lakatta, 1989). In fact, chronic treatment with mibefradil significantly reduced diastolic $[\text{Ca}^{2+}]_i$ in remodeled myocardium from postinfarction rats compared with placebo-, verapamil-, or amlodipine-treated animals. Moreover, ISO-stimulation induced diastolic Ca^{2+} oscillations in all papillary muscles from the placebo-, verapamil-, and amlodipine-MI group, whereas no such phenomena could be elicited in preparations from the mibefradil-MI or sham-operated group.

Experimental studies suggest that exposure to ISO induces an increase in T-type Ca^{2+} current secondary to a rise in $[\text{Ca}^{2+}]_i$ after augmentation of the L-type Ca^{2+} current (Tseng and Boyden, 1991). The threshold for opening of the T-type Ca^{2+} channel is lower than that of the L-type Ca^{2+} channel and a slight deviation from the resting potential may gener-

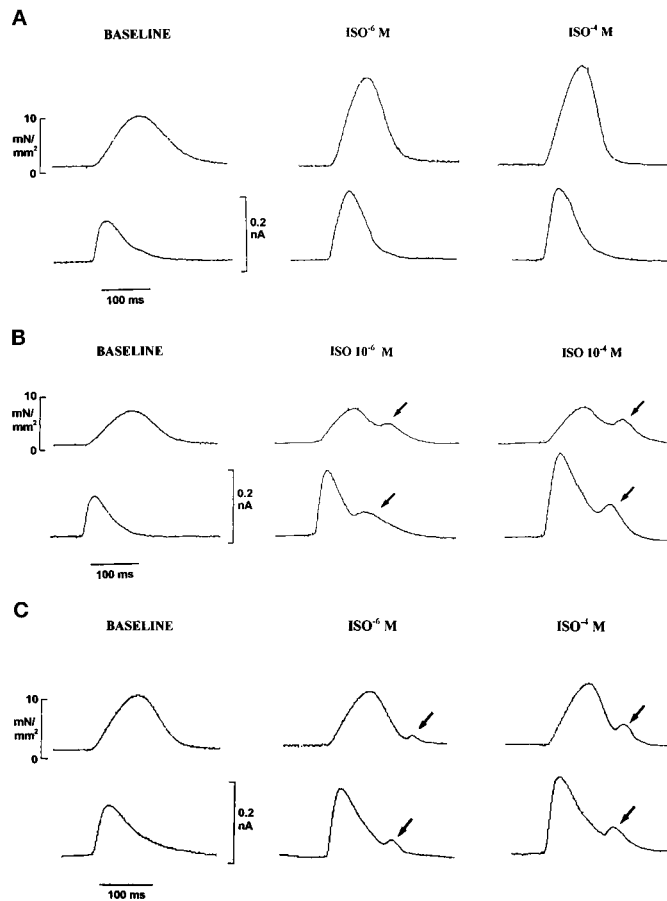


Fig. 3. Aequorin light signal and isometric contraction from representative rat papillary muscles of MI groups with mibefradil (A), verapamil (B), or amlodipine (C) treatment during ISO stimulation. Upper trace, isometric contraction; lower trace, light signal. Diastolic Ca²⁺ oscillations and mechanical aftercontractions are marked by arrows.

ate a depolarizing “window” current via the T-type Ca²⁺ channel which, in turn, could trigger spontaneous sarcoplasmic reticulum Ca²⁺ release, afterdepolarizations, and arrhythmias (Mishra and Hermesmeyer, 1994). According to this hypothesis, chronic treatment with mibefradil may have suppressed the activity of reexpressed T-type Ca²⁺ channels of remodeled rat myocardium in the present study. Thus, the resulting improvement of diastolic Ca²⁺ homeostasis induced a lowering of LVEDP, an increase in systolic and isometric force generation, and a reduced propensity for afterdepolarizations during ISO stimulation. The fact that peak systolic Ca²⁺ availability remained depressed in mibefradil-treated myocardium might be due to a concomitant partial blockade of L-type Ca²⁺ channels.

In summary, chronic T-type Ca²⁺ channel blockade with mibefradil proved to be more effective than conventional L-type Ca²⁺ channel blockade in preserving the hemodynamic, contractile and Ca²⁺, modulating function of remodeled myocardium from postinfarction rats. This finding points to the pathophysiological relevance of T-type Ca²⁺ channels in postischemic reperfused myocardium. The pharmacological mode of action of mibefradil might involve the myofibrillar Ca²⁺ responsiveness and a window current via T-type Ca²⁺ channels that should be addressed in further studies.

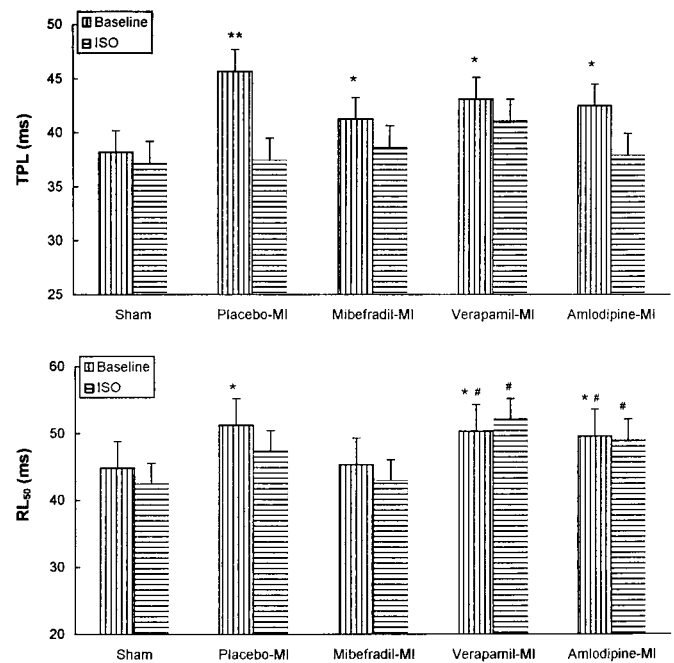


Fig. 4. Time intervals of the intracellular light transient at baseline and during ISO stimulation (10⁻⁴ M) in papillary muscles from sham-operated and myocardial infarction rats. Sham, sham-operated group; placebo-MI, MI group with placebo treatment; mibefradil-MI, MI group with mibefradil treatment; verapamil-MI, MI group with verapamil treatment; amlodipine-MI, MI group with amlodipine treatment ($n = 6$ in each group). TPL, time to peak light; RL₅₀, time from peak light to 50% decline. * $p < .05$, ** $p < .01$ versus sham-operated group; # $p < .05$ versus mibefradil-MI group.

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