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EFFECTS OF MIBEFRADIL, A BLOCKER OF T-TYPE Ca^{2+} CHANNELS, IN SINGLE MYOCYTES AND INTACT MUSCLE OF GUINEA-PIG HEART

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We investigated the effects of a relatively selective blocker of the T-type Ca^{2+} channels, mibefradil (MBF), in the isovolumic left ventricles of the isolated, perfused hearts of guinea-pigs and single myocytes isolated from the ventricles of this species. In the myocytes superfused with 0 Na^{+} solution containing 200 μM lidocaine and pulsed from −90 mV to −40 mV to +5 mV, MBF proved to be about 3 times more potent inhibitor of the T-type than of the L-type Ca^{2+} current. The effect on the L-type current was strongly voltage and use dependent. In the ventricles and in the myocytes contraction was reduced by 50% by about 1μM MBF, the concentration ~12 times higher than this increasing the coronary flow by 50%. In myocytes the decrease in unloaded shortening paralleled inhibition of the T-type rather, than of the L-type Ca^{2+} current. Inhibition of electrically stimulated contraction of the myocytes was three times stronger than inhibition of the caffeine contractures regarded as an index of sarcoplasmic reticulum (SR) Ca^{2+} content. These findings are consistent with the hypothesis that the T-type Ca^{2+} channels may contribute to release of Ca^{2+} from the SR. It is concluded that MBF has a definite negative inotropic effect in the ventricular myocardium of guinea-pig heart at the concentrations found in the blood of the patients submitted to the clinical trials.

K e y w o r d s: *mibefradil, heart muscle, cardiomyocytes, Ca^{2+} currents, excitation-contraction coupling.*

INTRODUCTION

Sarcolemma of cardiac myocytes is harbouring at least two types of Ca^{2+} channels: L (long lasting) type and T (transient) type. The current flowing through the L-type channels is believed to increase sarcoplasmic Ca^{2+} concentration over the threshold of the Ca^{2+} release channels of sarcoplasmic reticulum (SR) and to contribute to re-filling of SR depleted of its Ca^{2+} during cell excitation (1). In some species like guinea-pig and humans L-type current is

able to activate in ventricular myocytes a strong contraction with slow kinetics even when the SR function is blocked by thapsigargin or high ryanodine concentration (2—4) or due to inhibition of expression of its Ca^{2+}-ATPase in the failing heart (5). In guinea pig the mean maximal L-type current density is 4.7 ± 0.3 nA/nF (6). The T-type Ca^{2+} channels discovered for the first time in canine atrial myocytes by Bean (7) and in the ventricular myocytes of guinea-pig heart by Nilius *et al.* (8) are expressed to varying degree in various parts of the heart and in various species. It has been well documented that their expression is the highest in the embryonic and neonatal hearts in which the density of T- type current may be greater than that of L-type current and that it is progressively suppressed during maturation (9). In the adult hearts relatively strong T-type current has been reported in the cells of sino-atrial node (10, 11), in the atrial myocytes (7) and in the Purkinje fibres (12, 13). All these cells do not have the transversal T tubuls. In the ventricular myocytes the mean ratio of densities of T-current/L-current varies from 0 in young normal rats (14) or cats (15) to ~ 0.30 in guinea-pig (16). However, the ratio changes from cell-to-cell and some cells may have only the L-type channels. The T-type channels are reexpressed in the adult hearts in cardiomyopatic hamsters (17,18), in overload hypertrophy (15) or hypertrophy initiated by the growth hormone (19). These findings taken together with their high expression in the embryonic and neonatal hearts suggest that T-type Ca^{2+} channels are involved in the processes of growth. Their physiological role in the normal adult heart is still enigmatic. The T-type current is blieved to contribute to the slow diastolic depolarization in the cells of sino-atrial node (10,11). Their possible role in excitation-contraction coupling is still discussed. Recently it has been hypothesised that they may contribute to the release of Ca^{2+} from the SR as an additional source of Ca^{2+} activating the release channels (20,21) or as voltage sensors (22) similar to the L-type Ca^{2+} channels in the skeletal muscles. In this work we investigated the effects of a relatively selective blocker of the T-type Ca^{2+} channels, mibefradil (MBF) (23 and for review see 24) in the left ventricles of isolated, perfused hearts and in single isolated ventricular myocytes of guinea-pigs. Some results similar to ours have been already published by others. However, to our knowledge, inotropic and electrophysiological effects of MBF have never been together investigated in one study in the intact heart and in isolated cardiac myocytes. MBF has been withdrawn from the clinical trials when this work was nearly completed. However, hundreds of patients have been already treated with this drug and it is still an interesting experimental tool. We found that MBF in therapeutic concentrations has a significant negative inotropic effect in guinea-pig heart which parallels inhibition of the T-type current, rather, than inhibition of the L-type current.

METHODS

Intact isolated hearts

Guinea-pigs of both sexes weighing 200—250g were injected intraperitoneally with 2.500 U heparin followed by an overdose of pentobarbital sodium. The hearts were rapidly excised and perfused after the Langendorff method under the pressure of 85 mmHg with the Tyrode solution gassed with oxygen at 37°C. A latex balloon filled with Tyrode solution and connected with the metal cannula to the electromanometer was placed in the cavity of the left ventricle. The diastolic pressure was set at 10 mmHg. Intraballoon pressure and dP/dt was recorded with the Siemens-Elema multichannel chart recorder. Coronary flow was recorded by measuring volume of the heart effluent over 1 min. In 4 out of 8 experiments the sino-atrial node was crushed and the heart stimulated at the rate of 180/min with the bipolar electrodes attached to the right ventricle.

Single isolated cells

Guinea-pigs were heparinised and anaesthetised as described above. After the heart was rapidly excised and washed in the cold Tyrode solution, aorta was cannulated and retrogradely perfused for 5 min with nominally Ca^{2+} free Tyrode solution containing 10mM EGTA (ethylene glycol-bis-(-amino ethyl ether)-N,N,N',N'-tetraacetic acid) (for composition of the solutions see below). The initial perfusion period was followed by 20 min perfusion with Ca^{2+} free solution containing 20 mg collagenase B (Boehringer) and 3 mg protease (Sigma) per 50 ml. Thereafter the ventricles were minced with scissors in the same, enzyme containing solution to which 0.2 mM Ca^{2+} was added. Cell suspension was filtered through the nylon mash and cells were allowed to sediment. Thereafter they were washed with Tyrode solution containing 1.0 mM Ca^{2+}. Cells were placed in the 0.5ml superfusion chamber mounted on the stage of an inverted microscope (Nicon Diaphot) and superfused at the rate of ~2 ml/min. They were illuminated with the red (650—750 nm) light through the bright optics of the microscope. The microscope was equipped with the TV edge tracking system enabling continuous recording of the cell length. In other experiments cells were placed in the rapid superfusion system modified from Rich *et al.* (25) mounted on the stage of the separate TV edge tracking system devised and built by J. Palmer (Cardiovascular Laboratories, School of Medicine, UCLA, Los Angeles, USA). Cells were superfused by a stream of solution flowing between influx and efflux tubes (2mm diameter). The miniature magnetic valves directed to the influx tube solutions from one of the 4 containers. Complete exchange of the superfusing solutions took ~ 300 ms as checked by measurement of electrical resistance of the solution upon switching from distilled water to 100 mM NaCl.

The investigation conforms with the Guide for the Care and Use of the Laboratory animals published by the US National Institutes of Health (NIH Publication No 85-23, revised 1996).

Electrophysiological investigations

The ionic currents were recorded by the whole cell voltage clamp method. Pipettes of 2.5—3.6 MΩ resistance were pulled from borosilicate glass capillaries (Word Precision Instruments, USA). The cell membrane under the electrode was disrupted by negative pressure. In order to record the L-type Ca^{2+} current together with cell contractions we superfused the cells with normal Tyrode solution ([Ca^{2+} = 2 mM]) and the solution in the pipettes contained Na^{+}. Prepulses from a holding potential of −80mV to −40mV (150ms long) were applied in order to activate and inactivate the Na^{+} and T-type Ca^{2+} channels. They were followed by pulses to +5 mV

(200 ms long) which activated the L-type Ca^{2+} channels. In order to record the T-type and L-type Ca^{2+} channels the cells were superfused with 0 Na^+ solution and the internal solution did not contain Na^+. Prepulses from the holding potential of −90 mV to −40 mV (150 ms long) activated and inactivated the T-type Ca^{2+} channels. They were followed by pulses to +5mV which activated the L-type Ca^{2+} channels. For the reasoning justifying this procedure see Discussion. We used also the separate pulses from -90 mV to -40 mV activating T-type Ca^{2+} channels and from −90 mV to +5 mV activating both T- and L-type channels as well as pulses from the holding potential of −40 mV to +5 mV activating selectively the L-type channels.

Currents were measured with an Axopatch 1-D amplifier controlled by a personal computer using the V-clamp software and V-clamp interface generously offered by D.R. Matteson (Dept. of Biophysics, University of Maryland). Series resistance compensation was used to reduce voltage error due to the access resistance.

Solutions

External solutions:

1. Tyrode solution (in mM): 144 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 0.43 $NaHPO_4$, 10.0 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 11.0 glucose, 5.0 sodium pyruvate. The pH of the solution was adjusted with NaOH to 7.3 for cells isolation and to 7.4 for experiments. $CaCl_2$ was added as desired. $[Ca^{2+}]=2.0$ mM was used throughout experiments.

2. 0 Na^+ solution: 137.0 tetraethylammonium chloride (TEA-Cl), 10.0 CsCl, 5.4 KCl, 1.2 $MgSO_4$, 15.0 glucose, 20.0 HEPES, 2.0 $CaCl_2$ (after Bogdanov *et al*, 14) plus 200.0 μM lidocaine. pH was adjusted to 7.4 with KOH.

The pipette filling solutions:

1. Solution used with external Tyrode solution: 135.0 KCl, 2.0 $MgCl_2$, 8.0 NaCl, 10.0 HEPES, 5.0 Mg-ATP. pH was adjusted with KOH to 7.2.

2. Solution used with external 0 Na^+ solution: 100.0 CsCl, 20.0 TEA-Cl, 10.0 EGTA, 10.0 HEPES, 5.0 Mg-ATP. pH adjusted to 7.2 with KOH.

Protocols of experiments will be for clarity described in the Results section. The results are expressed as means ± SE.

RESULTS

The effect of mibefradil on left ventricular isovolumic contractions and coronary flow

The hearts were perfused at 37°C. They were beating spontaneously at the rate of 143—154/min (4 experiments) or the left ventricles were stimulated at the rate of 180/min (4 experiments). The unstimulated ventricles developed systolic pressure of 94±4 mmHg with the coronary flow of 106.4±4.7 ml/min/g w.w.The stimulated ventricles developed systolic pressure of 82±7 mmHg with the total coronary flow of 95.7±12.1ml/min/g w.w. *Fig. 1* shows the % dose-response of coronary flow and of isovolumic contractions to MBF. A 100 nM MBF increased coronary flow by ~65 %. At this concentration MBF decreased contractile force by ~15% in the spontaneously beating hearts and by ~45% in the stimulated hearts. Maximal increase of coronary flow by ~80% was attained at 500 nM concentration of MBF which decreased the contractile force in the spontaneously beating hearts by ~30% and in the

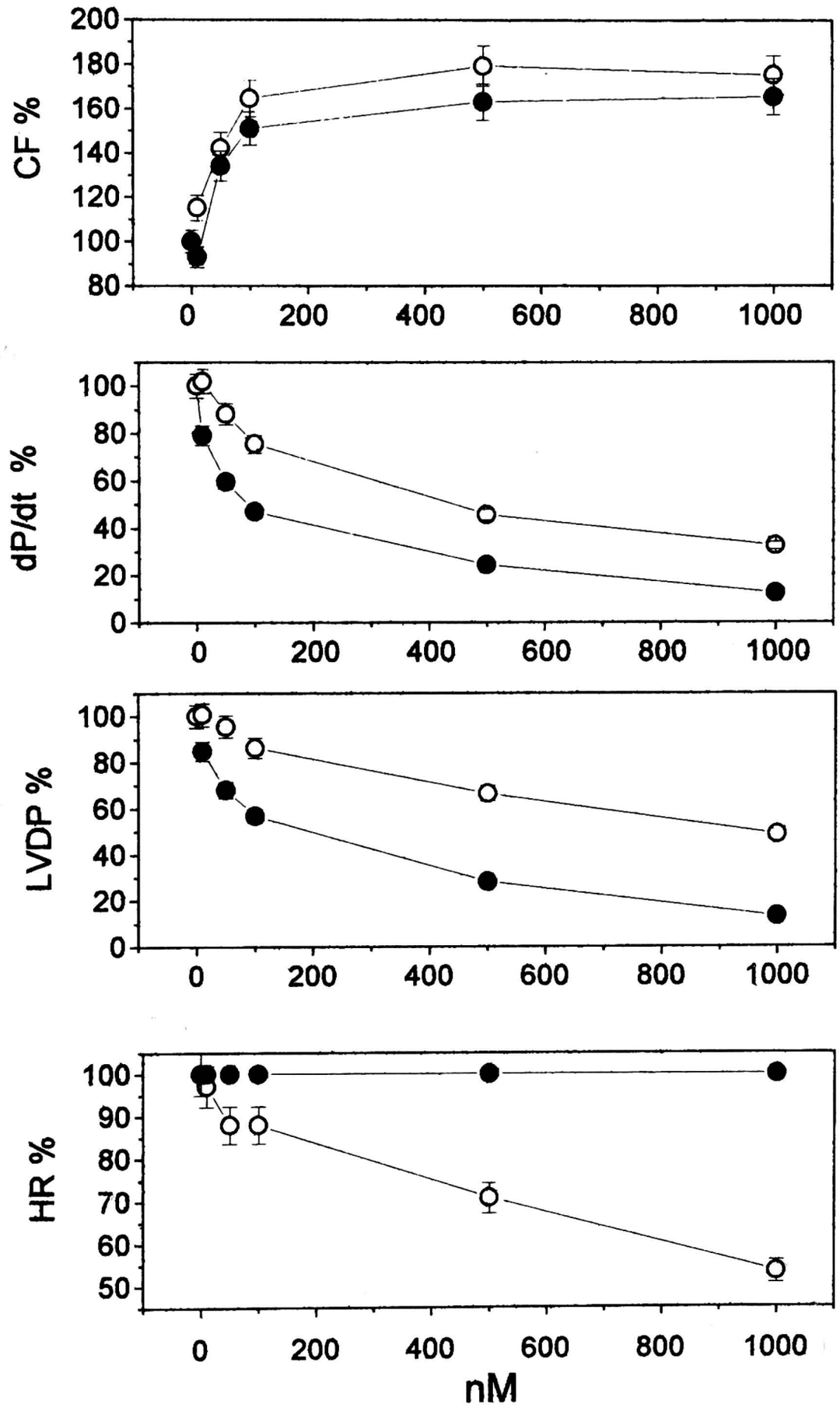

Fig. 1. The effect of mibefradil on coronary flow (CF), isovolumic left ventricular developed pressure, (LVDP) dP/dt, and heart rate (HR) of isolated guinea-pig hearts. Ordinates: % of control. Open symbols: spontaneously beating hearts (n = 4). Filled symbols: the hearts stimulated at the rate of 180/min (n = 4). Means ± SE.

stimulated hearts by ~50%. About 50% and 70% decrease of contractile force was caused by 1000 nM MBF, respectively. These results are similar to those obtained in the isovolumic left ventricles of guinea-pigs by Osterrieder and Holtz (26).

Ca^{2+} currents in single myocytes

In cells superfused with 0 Na^+, lidocaine containing solution, voltage steps from −90mV to −40mV initiated the rapidly inactivating inward currents of small amplitude which were not inhibited by 20 μM nifedipine but were blocked by 250 μM Ni^{2+} (*Fig. 2*, top panel). The following steps from −40 to

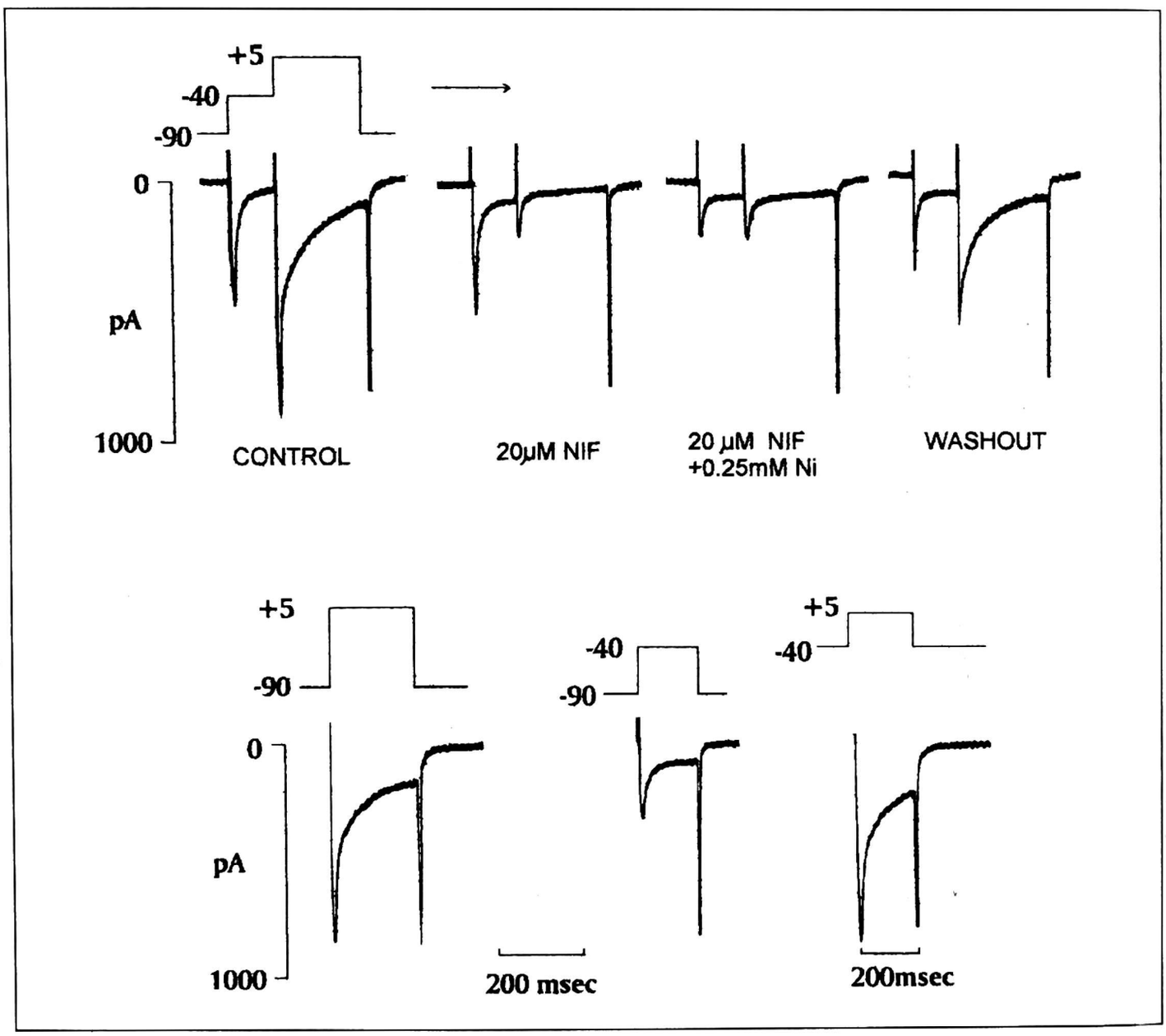

Fig. 2. The membrane currents of the single ventricular myocytes of guinea-pig heart superfused with 0 Na^+ solution containing 200 μM lidocaine. Voltage protocols shown above the current records. Top panel: the effects of nifedipine (NIF) and Ni^{2+}. Bottom panel: the effects of holding and test potentials.

+5 mV initiated slowly inactivating inward currents of large amplitude which were blocked by 20 μM nifedipine (*Fig. 2*, top panel). Single pulses from −90 or −40 to +5mV initiated slowly inactivating currents of large amplitude whereas pulses from −90 to −40 mV initiated rapidly inactivating currents of low amplitude. (*Fig. 2*, bottom panel). Considering the specific sensitivity of the currents to Ni^{2+} and nifedipine and range of activating and inactivating voltages (27) we infer that the pulses from −90 mV or −40 mV to +5 mV activated L-type channels whereas the pulses from −90 to −40mV activated selectively T-type channels. The ratio of maximal intensity of T-type to L-type currents was 0.21 ± 0.07 (range 0.03-0.7).

Effect of mibefradil on Ca^{2+} currents and contractions of single cells

Fig. 3 illustrates selectivity of MBF as a blocker of T-type current: in this cell 1μM MBF reduced the T-type current by ~50% without any effect on the L-type current. *Fig. 4* shows the concentration dependence of blocking of currents of both types. It is clear that MBF affects much stronger the T-type channels than the L-type channels (*Fig. 4*). This result is consistent with that of Mishra and Hermsmeyer (28), although in our experiments the difference between the effect on T- and L-type currents is less.

The effect of MBF on the L-type current was strongly voltage and use dependent. *Fig. 5(1)* shows the L-type current elicited in the presence of 1.0μM MBF by the last of 20 pulses from a holding potential of −80 mV to +5 mV. Thereafter pulsing was stopped for 2 min and the holding potential was

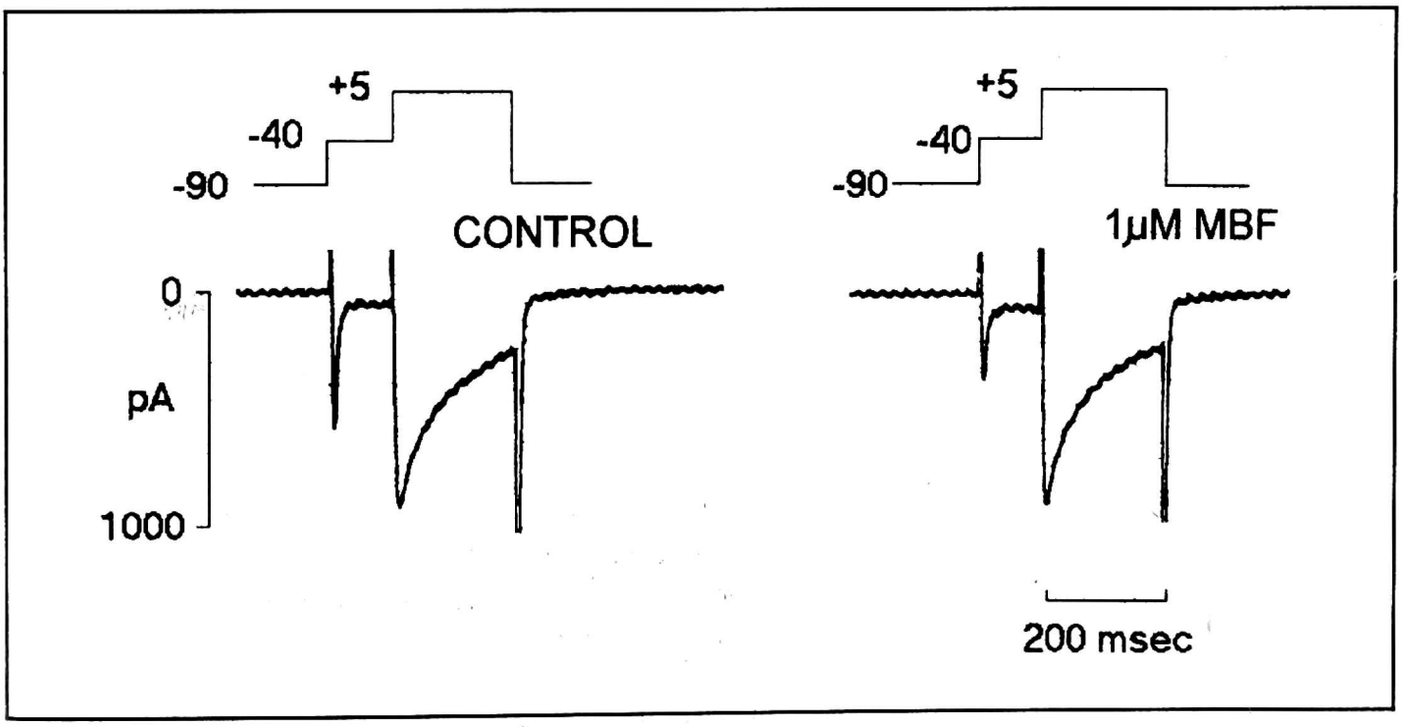

Fig. 3. The effect of mibefradil (MBF) on membrane currents of single ventricular myocyte of guinea-pig heart superfused with 0 Na^+ solution containing 200 μM lidocaine. Voltage protocols shown above the current records.

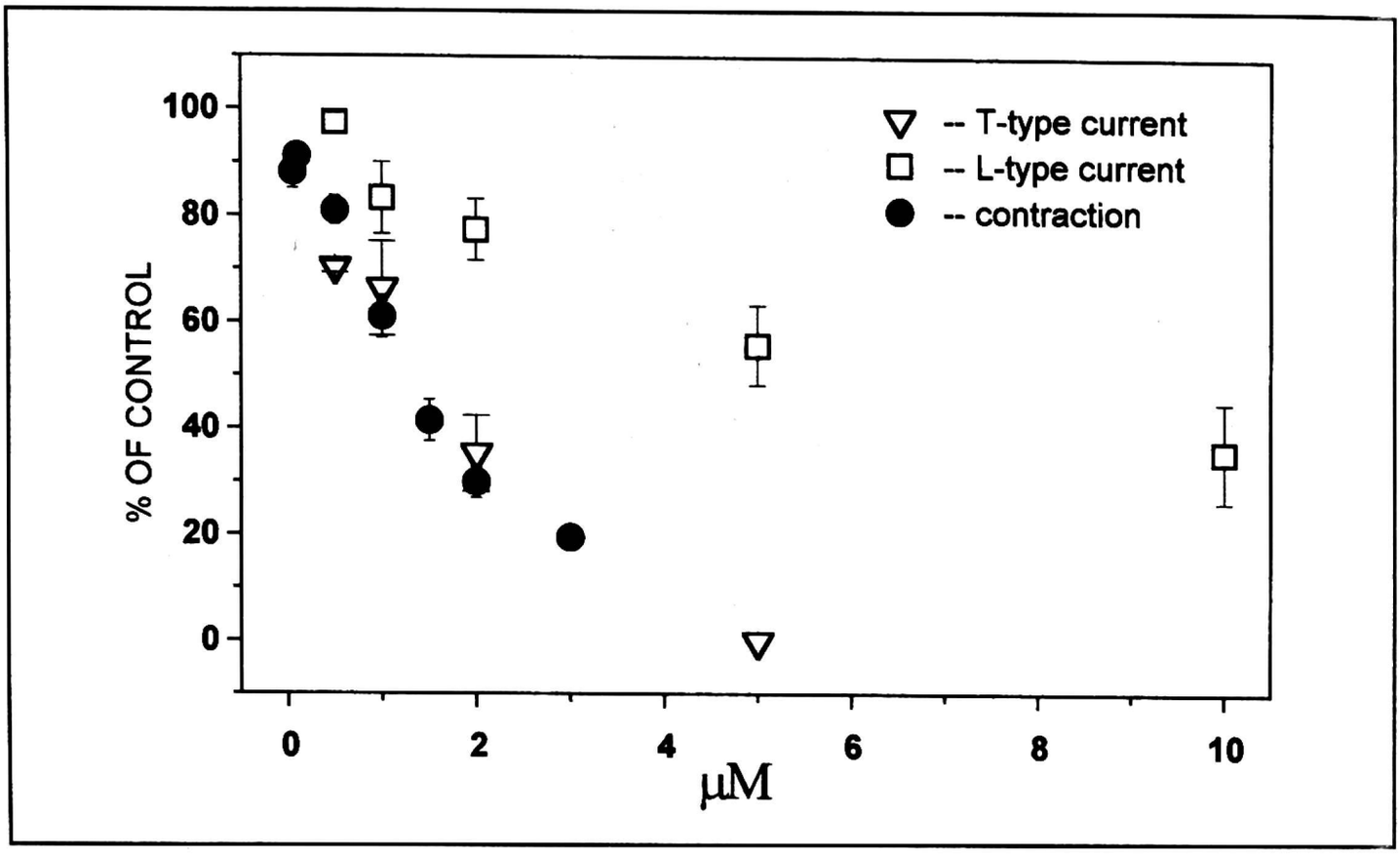

Fig. 4. The effect of mibefradil on the L-type and T-type Ca^{2+} currents and contractions of single left ventricular myocytes of guinea-pig heart. Means ± SE, n = 15 (contractions) and 5—8 (currents). Contractions and the currents recorded in separate experiments.

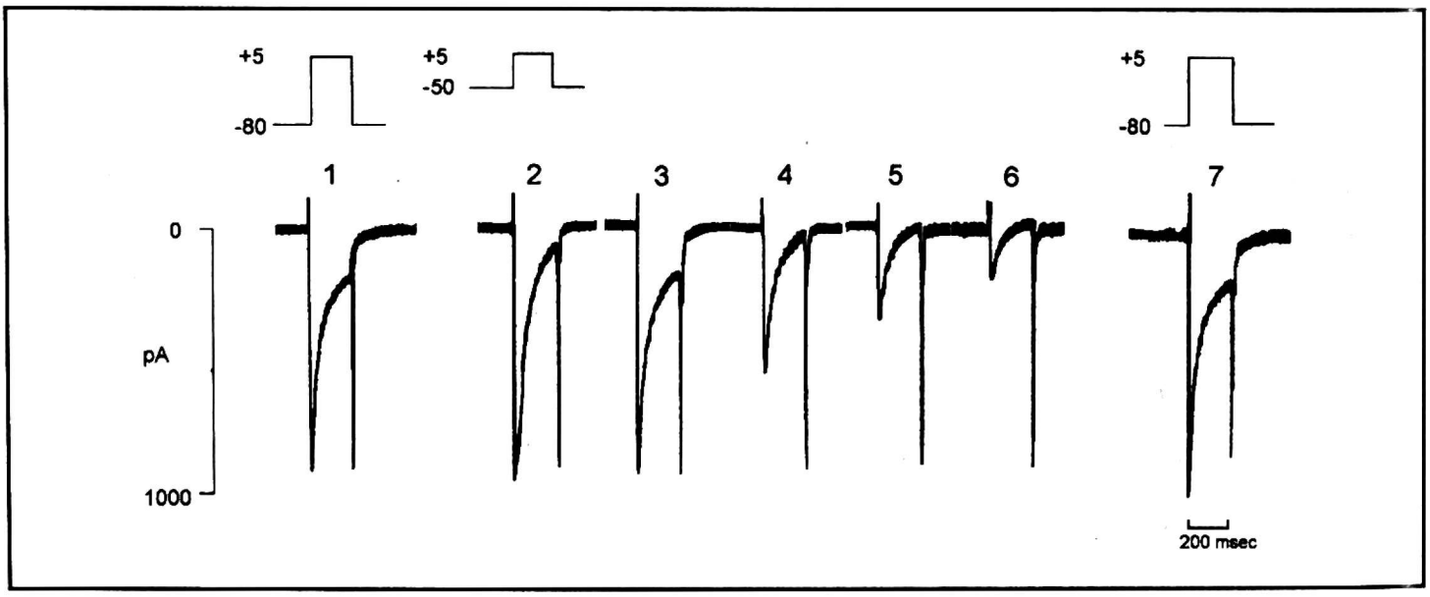

Fig. 5. Voltage and use dependence of the effect of mibefradil on the L-type Ca^{2+} current of a single ventricular myocyte of guinea-pig heart superfused with 0 Na^{+}solution containing 200 μM lidocaine and 1.0 μM mibefradil. 1: last of the 20 pulses at the rate of 30/min. Two min break in stimulation between 1 and 2. Holding potential switched from −80 to −50 mV at the beginning of the pause. 2 through 6: successive pulses at the rate of 30/min. Holding potential switched from −50 to −80 mV at the beginning of 15 sec pause between 6 and 7.

switched to −50 mV at the very beginning of the pause. *Fig. 5(2)* shows that change in membrane potential alone did not affect the current. However, the following pulsing to +5 mV at the rate of 30/min resulted in progressive inhibition of the current. After (6) the holding potential was again switched to

−80 mV and after 15 sec break the cell was pulsed to +5 mV (7). Fifteen sec repolarization to −80 mV removed the inhibition. This result confirms the voltage dependence of the effect of MBF on the L-type channels reported by Fang and Osterrieder (29) and stresses the very strong use dependence.

In single cells superfused with Tyrode solution and field stimulated at the rate of 30/min, MBF in the lowest concentrations tested (0.05μM) had statistically significant negative inotropic effect. The 1μM MBF reduced the unloaded cell shortening by ~40%. *Fig. 4* shows that negative inotropic effect of MBF parallels inhibitory effect on the T-type currents, rather, than its effect on the L-type currents. On the other hand, Ca^{2+} influx with the L-type current is many fold larger than that with the T-type current. Therefore it may be argued that small % reduction of the L-type current may limit the Ca^{2+} influx more than larger % reduction of the T-type current. Thus the negative inotropic effect of MBF could, in fact, result from inhibition of the L-type current. It should be also considered that the field stimulated cells could have variable resting potentials, some of them being slightly depolarized. Therefore MBF could inhibit their L-type current more than in the voltage clamped cells. However, as shown in Fig.6, MBF could decrease the cell shortening without any affect on the L-type current. Similar result was obtained in 4 other cells.

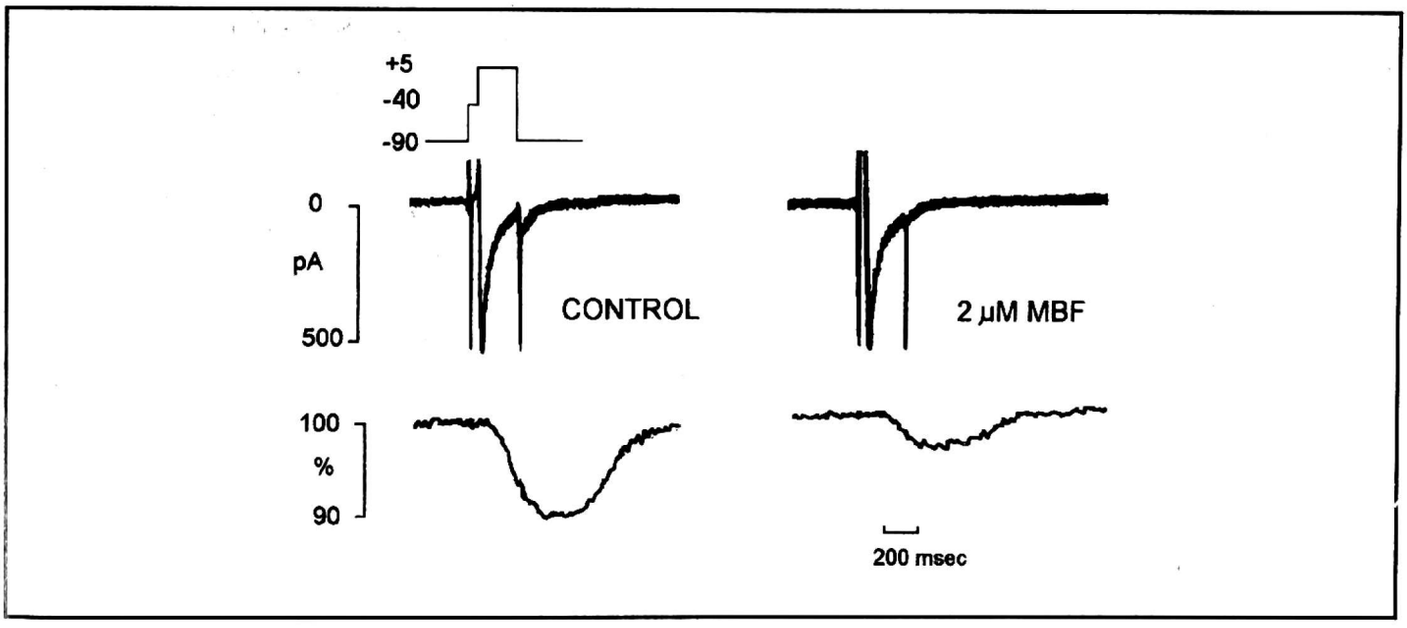

Fig. 6. Effect of mibefradil (MBF) on the L-type Ca^{2+} current (top) and contractions of a single ventricular myocyte of guinea-pig heart superfused with the normal Tyrode solution and pulsed at the rate of 30/ min. The bottom scale — % of the resting cell length. Voltage protocol pertains to both records.

The effect of mibefradil on the SR Ca^{2+} content

The above experiments suggest that MBF has a negative inotropic effect in the concentrations which inhibit significantly the T-type Ca^{2+} current but have little effect on the L-type current. Mishra and Hermsmeyer (28) reported that MBF might affect the Ca^{2+} release from SR of vascular smooth muscle cells.

Recently it has been proposed that T-type Ca^{2+} channels may contribute to Ca^{2+} induced Ca^{2+} release from the SR of ventricular myocytes (20, 21) or play a role similar to the voltage sensors (22) in skeletal muscle. Therefore in the next series of experiments we compared in the same cells the effects of MBF on their contractile responses to the rapid superfusion of 15mM caffeine with its effects on electrically stimulated contractions. Caffeine activates the Ca^{2+} release channels of the SR and the resulting contraction is currently used as a relative index of the SR Ca^{2+} content. Cells were field stimulated at the rate of 30/min. When the steady state of contractions was attained in control runs or under the effect of a given MBF concentration, stimulation was stopped and 15 mM caffeine superfused for 15 sec. Thereafter stimulation was resumed. Caffeine superfusion stimulated contracture the amplitude of which attained $127 \pm 4\%$ of that of electrically stimulated contractions. Amplitude of contractions stimulated after caffeine superfusion was reduced by $\sim 80\%$. The pre-caffeine amplitude recovered over 4—6 following beats (not shown). As shown in *Fig. 7* the negative effect of MBF on the electrically stimulated contractions was at least three times stronger than that on the caffeine stimulated contractions. Three μM MBF reduced the electrically stimulated contraction by 75% whereas the ceffeine contracture was reduced only by 35%. This result suggests that MBF decreased the fraction of Ca^{2+} released from the SR upon the sarcolemmal depolarization.

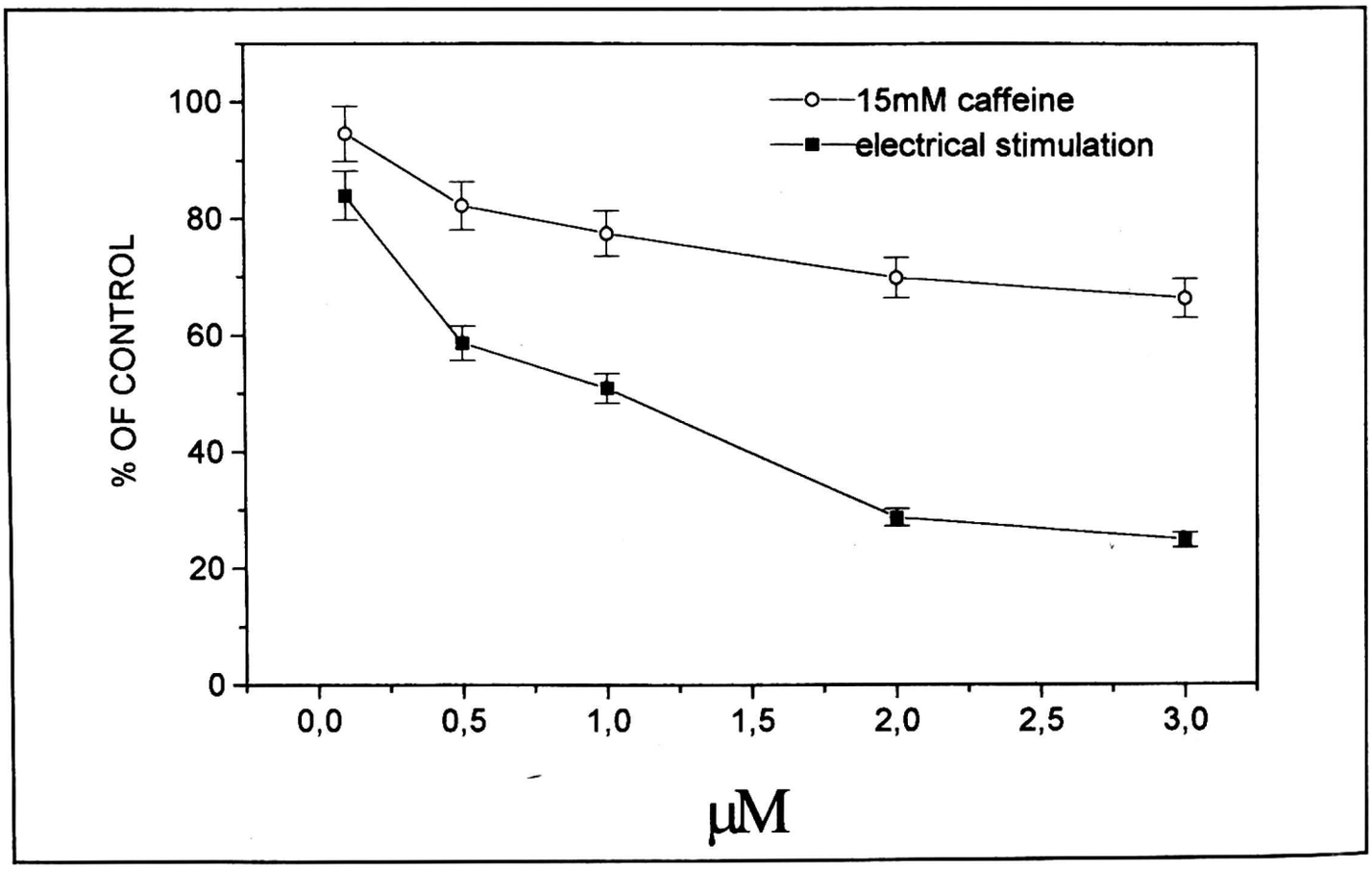

Fig. 7. The effect of mibefradil on amplitude of caffeine contractures and electrically stimulated contractions of isolated ventricular myocytes of guinea-pig hearts superfused with Tyrode solution and paced at 30/min. Means ± SE, n = 12.

DISCUSSION

To our knowledge our paper for the first time describes the effects of MBF on coronary circulation and contractility of the intact heart of guinea pig and on the membrane currents and contractions of single isolated cardiomyocytes of the same species investigated in one study. The main finding of this work is that MBF has a significant negative inotropic effect in intact left ventricular myocardium as well as in single isolated myocytes of guinea-pigs in concentrations attained in blood of patients submitted to the clinical trials (24). This effect parallels inhibition by MBF of the T-type Ca^{2+} current. The negative inotropy of MBF was stronger in the paced ventricles than in the ventricles of spontaneously beating hearts. This difference may result from the inverse relationship between the amplitude of the T-type Ca^{2+} current and the rate of stimulation (16 and our unpublished data).

For investigation of the effect of MBF on the T-type and L-type Ca^{2+} currents we used the double step pulses: the first step from the holding potential of -90 mV to -40 mV and the second from -40 mV to $+5$ mV. This approach has not been used by other authors investigating the T-type Ca^{2+} channels, however, it seems justified for the following reasons. The T-type channels inactivate within ~ 60 msec (16 and this study). The duration of the first step to -40 mV was 150 ms. So the T-type current was inactivated before the second step to $+5$ mV was applied and it should not have contaminated the L-type current. The effect of MBF on the L-type current depends strongly on the holding potential (29 and this study, *Fig. 5*). So it could be argued that switching of the holding potential to -40 mV before activation of the L-type current increases the inhibitory effect of MBF on the L-type channels over that in the not clamped cell whose resting potential is between -80 and -70 mV. However, we found that switching of the holding potential from -90 mV to -40 mV in the presence of MBF does not affect the L-type current for 2 min if the cell is not stimulated (*Fig. 5*). So switching the holding potential from -90 to -40 mV for 150ms should not have increased the inhibitory effect of MBF on the L-type current. We found that the inhibitory affect of MBF on the L-type current at less negative holding potential is strongly use-dependent (*Fig. 5*). However, we also found that inhibition is almost instantaneously removed by repolarization. So our holding potential of -90 mV between the pulses should have removed the effect of the preceding pulse and prevent its cumulating during steady state pulsing.

We found that negative inotropic effect of MBF parallels % inhibition of T-type Ca^{2+} current, rather, than inhibition of the L-type current. It may be argued that Ca^{2+} influx by L-type channels is manifold larger than that through the T-type channels. Thus a given % inhibition of L-type current would decrease the Ca^{2+} influx much more than the same % inhibition of the

T-type current. However, in some cells in which we recorded contractions and the L-type current, 2 μM MBF strongly reduced contractions without any effect on the current (*Fig. 6*). Our results might suggest that T-type channels significantly contribute to activation of contraction. This would be consistent with a hypothesis that T-type Ca^{2+} channels contribute to the Ca^{2+} release from the SR either as a source of Ca^{2+} activating the release channels of the SR (20, 21) or as the voltage sensors (22) similar to the L-type Ca^{2+} channels in skeletal muscles. We found that the decrease in electrically stimulated contraction under the effect of MBF is three times larger than decrease in cell's contractile response to caffeine (index of the SR Ca^{2+} content). This suggests that one of the mechanisms of the negative inotropic effect of MBF could be reduced release of Ca^{2+} from the SR upon cell depolarization rather, than decrease in the SR Ca^{2+} content, also consistent with the above hypothesis.

The inotropic effects of MBF reported by other authors are variable and apparently depend on animal species and conditions of experiments. In isolated hearts of guinea pigs 0.05 μM MBF increased the coronary flow by 50% but only 14μM MBF halved the force of contraction, its inotropic effect being about ten times less potent then that of verapamil (26). In our experiments with the intact isolated hearts of guinea-pigs similar concentration of MBF doubled the coronary flow but already 1 μM halved the contractile force. In isolated, blood perfused papillary muscles of the dog heart amount of MBF that reduced the force of contraction by half was above 10 times the concentration that doubled blood flow (30). In isolated canine cardiac myocytes 1 μM MBF reduced the amplitude of Ca^{2+} transients by 25% (31). Since fluorescence — $[Ca^{2+}]_i$ relation is not linear, the inotropic effect could be even stronger. MBF did not reduce contractility of the hearts in situ of the rats without and with the heart failure (30). In isolated, electrically driven streaps of the left ventricular papillary muscles of the failing human hearts MBF decreased the basal force of contraction only in concentrations higher than 10μM (33). These differences apparently depend on the animal species. For example the rats do not show the T-type Ca^{2+} current in their ventricular myocytes (14) which may account for the lack of negative inotropic effect of MBF in their hearts. On the other hand the differences may very much depend on the not always controlled condition of the tested preparation. Decrease in the resting potential would enforce the inhibition of the L-type Ca^{2+} channels by MBF thereby increasing its negative inotropic effect.

In conclusion, we found that MBF has a definite negative inotropic effect in the guinea-pig hearts at the concentrations found in the blood of patients submitted to the clinical trials and that the effect parallels inhibition by MBF of the T-type Ca^{2+} channels. It is very likely that this negative inotropy would be increased in partly depolarized cells driven at the high rate due to the strong dependence of the inhibitory effect of MBF on the L-type Ca^{2+} channels on the membrane potential and its use dependence.

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REFERENCES

1. Langer GA. Excitation-contraction coupling and calcium compartmentation. In: The Myocardium, G.A. Langer (ed), New York, Academic Press, 1997, pp. 181—233.
2. Lewartowski B, Wolska B. The effect of thapsigargin on sarcoplasmic reticulum Ca^{2+} content and contractions in single myocytes of guinea-pig heart. *J Mol Cell Cardiol* 1993; 25: 23—29.
3. Lewartowski B, Rózycka M, Janiak R. Effects of thapsigargin in normal and pretreated with ryanodine guinea-pig cardiomyocytes. *Am J Physiol* 1994; 266: H1829-H1839.
4. Janiak R, Lewartowski B, Langer GA. Functional coupling between sarcoplasmic reticulum and Na/Ca exchange in single myocytes of guinea-pig and rat heart. *J Mol Cell Cardiol* 1996; 28: 253—264.
5. Davia K, Davies CH, Harding SE. Effects of inhibition of sarcoplasmic reticulum calcium uptake on contraction in myocytes isolated from failing human ventricle. *Cardiovasc Res* 1997; 33: 88—97.
6. Rose WC, Balke W, Wier WG, Marban E. Macroscopic and unitary properties of physiological ion flux through L-type Ca^{2+} channels in guinea-pig heart cells. *J Physiol* (*Lond*) 1992; 456: 267—284.
7. Bean BP. Two kinds of calcium channels in canine atrial cells. Differences in kinetics, selectivity and pharmacology. *J Gen Physiol* 1985; 86: 1—30.
8. Nilius B, Hess P, Lansman JB, Tsien RW. A novel type of cardiac calcium channel in ventricular cells. *Nature* 1985; 316: 443—446.
9. Rochwedel J, Maltsev V, Bober E, Arnold HH, Hescheler J, Wobus AM. Muscle cell differentiation of embrionic stem cells reflects myogenesis *in vivo*: developmentally regulated expression of myogenic determination genes and functional expression of ionic currents. *Dev Biol* 1994; 164: 87—101.
10. Hagiwara N, Irisawa H, Kameyama M. Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. *J Physiol* (*Lond*) 1988; 395: 233—253.
11. Weiss JN. Ion channels in cardiac muscle. In: The Myocardium, GA Langer (ed) New York, Academic Press, 1997, pp. 81—136.
12. Hirano Y, Fozzard HA, January CT. Characteristics of L- and T-type Ca^{2+} currents in canine cardiac Purkinje cells. *Am J Physiol* 1989; 256: H1478-H1492.
13. Tseng GN, Boyden PA. Different effects of intracellular Ca^{2+} and protein kinase C on cardiac T and L Ca^{2+} currents. *Am J Physiol* 1991; 261: H364-H379.
14. Bogdanov KY, Ziman BD, Spurgeon HA, Lakatta EG. L- and T-type calcium currents differ in finch and rat ventricular cardiomyocytes. *J Mol Cell Cardiol* 1995; 27: 2581—2593.
15. Nuss HB, Houser SR. T-type Ca^{2+} current is expressed in hypertrophied adult feline left ventricular myocytes. *Circ Res* 1993; 73: 777—782.
16. Mitra R, Morad M. Two types of calcium channels in guinea pig ventricular myocytes. *Proc Natl Acad Sci USA* 1986; 83: 5340—5344.
17. Sen L, Smith TW. T-type Ca^{2+} channels are abnormal in geneticaly determined cardiomyopathy hamster hearts. *Circ Res* 1994; 75:149—155.

18. Bkaily G, Sculptoreanu A, Jacques D, Jasmin G. Increases of T-type Ca^{2+} current in heart cells of cardiomyopatic hamster. *Mol Cell Biochem* 1997; 176: 199—204.
19. Xu X, Best PM. Increase in T-type calcium current in atrial myocytes from adult rats with growth hormone secreting tumors. Proc Natl Acad Sci USA 1990; 87: 4655—4659.
20. Sipido KR, Carmeliet E, Vanderwerf F. T-type Ca^{2+} current as a trigger for Ca^{2+} release from the sarcoplasmic reticulum in guinea-pig ventricular myocytes. *J Physiol (Lond)* 1998; 508: 439—451.
21. Zhou ZF, January CT. Both T- and L-type Ca^{2+} channels can contribute to excitation-contraction coupling in cardiac Purkinje cells. *Biophys J* 1998; 74: 1830—1839.
22. Howlett SE, Zhu JQ, Ferrier GR. Contribution of a voltage-sensitive calcium release mechanism to contraction in cardiac ventricular myocytes. *Am J Physiol* 1998; 274: H155-H170.
23. Ertel EA. Mibefradil (Ro 40—5967) is a selective blocker of myocardial T-type vs. L-type Ca^{2+} channels. *Biophys J* 1996; 70(2): A315.
24. Abernethy DR. Pharmacologic and pharmacokinetic profile of mibefradil, a T- and L-type calcium channel antagonist. *Am J Cardiol* 1997; 80(4B): 4C-11C.
25. Rich TL, Langer GA, Klassen MG. Two components of coupling calcium in single ventricular cells of rabbits and rats. *Am J Physiol* 1988; 254: H937 — H946.
26. Osterrieder W, Holck M. In vitro pharmacologic profile of Ro 40—5967, a novel Ca^{2+} channel blocker with potent vasodilator but weak inotropic action. *J Cardiovasc Pharmacol* 1989; 13: 754—759.
27. Balke CW, Rose WC, Marban E, Wier WG. Macroscopic and unitary properties of physiological ion flux through T- type Ca^{2+} channels in guinea-pig heart cells. *J Physiol (Lond)* 1992; 456: 247—265.
28. Mishra SK, Hermsmeyer K. Selective inhibition of T-type Ca^{2+} channels by Ro-40-5967. *Circ Res* 1994; 75: 144—148.
29. Fang LM, Osterrieder W. Potential-dependent inhibition of cardiac Ca^{2+} currents by Ro 40—5967 and verapamil: relation to negative inotropy. Eur J Pharmacol 1991; 196: 205—207.
30. Orito K, Satoh K, Taira N. Cardiovascular profile of Ro 40—5967, a new nonhydropyridine calcium antagonist, delined in isolated, blood perfused dog hearts. *J Cardiovasc Pharmacol* 1993; 22: 293—299.
31. Hensley J, Billman GE, Johnson JD, Hohl CM, Altschuld RA. Effects of calcium channel antagonists on Ca^{2+} transients in rat and canine cardiomyocytes. *J Mol Cell Cardiol* 1997; 29,1037—1043.
32. Clozel JP, Veniant M, Osterrieder W. The structuraly novel Ca^{2+} channel blocker Ro 40—5967, which binds to the (3H) desmethoxyverapamil receptor, is devoid of the negative inotropic effect of verapamil in normal and failing rat hearts. *Cardiovasc Drugs Ther* 1990; 4(3): 731—736.
33. Cremers B, Flesch M, Sudkamp M, Bohm M. Effects of the novel t-type calcium channel antagonist mibefradil on human myocardial contractility in comparision with nifedipine and verapamil. *J Cardiovasc Pharmacol* 1997; 29: 692—696.

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