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Potent vasodilatory with minor cardiodepressant actions of mibefradil in human cardiac tissue

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1 The present study compared the cardiovascular effects of mibefradil (MIB), a novel Ca^{2+}-channel antagonist with high selectivity for T-type Ca^{2+}-channels to the effect of the L-type Ca^{2+}-channel-antagonists nifedipine (NIF) and diltiazem (DIL) in left ventricular myocardium and coronary arteries of hearts obtained from patients suffering from dilated cardiomyopathy (NYHA IV). Right atrial myocardium from patients undergoing aortocoronary bypass surgery without signs of cardiac failure was studied as well.

2 NIF and DIL (100 μmol l^{-1}) completely depressed force of contraction (FOC) in electrically driven left ventricular myocardium (NIF $6.5 \pm 1.4\%$ and DIL $7.1 \pm 1.2\%$ of control), whereas a similar concentration of MIB only reduced force of contraction to $55.1 \pm 4.0\%$ of the basal FOC. The negative inotropic potency as measured by the concentration needed to reduce basal FOC for 25% was NIF (0.0095 μmol l^{-1}) > DIL (0.041 μmol l^{-1}) > MIB (9.47 μmol l^{-1}).

3 All three Ca^{2+}-channel antagonists were more potent in human atrial compared to human left ventricular myocardium to reduce FOC.

4 The rank order of Ca^{2+}-antagonistic moiety as measured by the decrease of the intracellular Ca^{2+}-transient (fura-2 ratio method) was NIF > DIL > MIB.

5 All Ca^{2+}-channel antagonists completely relaxed human coronary arteries (% of papaverine effect: MIB $81.7 \pm 5.5\%$, DIL $91.3 \pm 0.9\%$, NIF $96.4 \pm 3.7\%$) precontracted with $PGF_{2\alpha}$ (0.3 μmol l^{-1}). The rank order of vasodilatory potency was NIF (EC_{50}; 0.02 μmol l^{-1}) > DIL (0.13 μmol l^{-1}) > MIB (2.05 μmol l^{-1}).

6 The vasoselectivity measured by the ratio of the concentration needed to achieve a 25% decrease in force and the concentration needed for 25% vasodilatation was 316 for MIB, 1.5 for NIF and 1.0 for DIL.

7 The present study provides evidence that blockade of T-type Ca^{2+}-channels (e.g. mibefradil) results in potent vasodilatory properties with only minor cardiodepressant effects.

Keywords: T-type Ca^{2+}-channel; L-type Ca^{2+}-channel; mibefradil; nifedipine; diltiazem

Introduction

Ca^{2+}-channel antagonists develop their cardiovascular actions by blocking the Ca^{2+}-entry through specific voltage-dependent Ca^{2+}-channels of the sarcolemmal membrane. In cardiac and vascular myocytes two types of Ca^{2+}-channels were found, T- and L-type Ca^{2-} channels (Bean, 1985; Nilius *et al*., 1985; Mitra & Morad, 1986; Beuckelmann, 1997). The T-type Ca^{2+}-channel is characterized by a low threshold potential and a short Ca^{2+} inward current. Upon stimulation, these characteristics result in a very rapid inactivation of the T-type Ca^{2+}-channel. In contrast, the threshold potential of the L-type Ca^{2+}-channel is higher than that of the T-type Ca^{2+}-channel. Furthermore, a longer time is required for the inactivation of the L-type Ca^{2+}-channel. The L-type Ca^{2+}-channel can be inhibited by Ca^{2+}-channel antagonists of the 1,4-dihydropyridine type, for example nifedipine (Bean *et al*., 1986). In high concentrations (3 μmol l^{-1}) 1,4 dihydropyridines can inhibit the T-type Ca^{2+}-channels as well (Bean, 1985; Nilius *et al*., 1985; Mitra & Morad, 1986; Hagiwara *et al*., 1988). Selective inhibitory responses for the T-type Ca^{2+}-channel have been shown for the insecticide tetramethrin (Hagiwara *et al*., 1988).

Because of their beneficial vasodilatory effects, Ca^{2+}-antagonists are used in the treatment of stroke disease, hypertension (Snyder & Reynolds, 1985), and coronary heart disease (McGrath *et al*., 1989). The long acting Ca^{2+}-antagonist amlodipine has been shown to exert beneficial effects in the long-term treatment of patients with heart failure due to dilated cardiomyopathy (PRAISE-study, O'Connor *et al*., 1995). However, the use of Ca^{2+}-antagonists may be limited by their negative inotropic actions, leading to reflex-induced tachycardia, especially in patients with an already comprised cardiac function. A negative inotropic effect of Ca^{2+}-channel-antagonists was shown in animal models *in vitro* (Boyd *et al*., 1988) and on isolated left ventricular and right atrial trabeculae from human heart (Schwinger *et al*., 1990a,b). This negative inotropic action of Ca^{2+}-channel antagonists has been demonstrated in myocardium of patients with moderate and severe heart failure (Schwinger *et al*., 1990a,b). *In vivo*, the negative inotropic action of Ca^{2+}-channel antagonists may be masked by the compensatory action of the sympathetic nervous system.

In patients with decreased cardiac function, the regulation of the intracellular Ca^{2+}-homeostasis is impaired (Gwathmey *et al*., 1987; Beuckelmann *et al*., 1992; Schwinger *et al*., 1995). In addition, the Ca^{2+}-sensitivity of the contractile proteins

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may be enhanced (Schwinger *et al*., 1994; Wolff *et al*., 1996). In this respect, a further reduction of the systolic Ca^{2+}-availability may be detrimental. Pieske *et al*. (1995) demonstrated that the negative force-frequency relationship in failing human myocardium is linked with a blunted systolic Ca^{2+}-increase in response to an enhanced stimulation frequency. Consequently, Ca^{2+}-antagonists exerting potent vasodilatation with no or only minor negative inotropic effects might be advantageous.

Mibefradil (Ro 40-5967), a substituted tetralin derivative ((1S,2S) - 2 - [2 - [[3 - (2 - benzimindazolyl) -propyl]methylamino] ethyl] - 6 - fluroro - 1,2,3,4 - tetrahydro - 1 - isopropyl - 2 -naphtyl methoxyacetate dihydrochloride), is a newly developed Ca^{2+}-channel antagonist, which blocks T-type Ca^{2+}-channels in vascular smooth muscle (Mishra & Hermsmeyer, 1994). In animal studies, a minor cardiodepressive effect of mibefradil was described (Clozel *et al*., 1990, 1991; Véniant *et al*., 1991). The occurrence of T-type Ca^{2+}-channels in cardiac tissue seems to be species-dependent. It has been found in the guinea-pig (Mitra & Morad, 1986) and finch heart (Bogdanov *et al*., 1995), but not in the rat (Bogdanov *et al*., 1995) and in human cardiac myocytes (Beuckelmann *et al*., 1991; Ouadid *et al*., 1991). In addition, the occurrence of T-type Ca^{2+}-channels seems to be age-dependent (Xu & Best, 1992) and may increase when certain conditions, e.g. hypertrophy (Nuss & Houser, 1993), act on the heart. Therefore, the use of human cells in experimental studies is important for at least two reasons: (1) the identification of species-differences, and (2) the analysis of human cardiac pathologies which are not reproducible in animals (Nargeot *et al*., 1997).

The present study was designed to investigate the vasodilatory and inotropic effects of mibefradil in comparison to the L-type Ca^{2+} channel antagonists nifedipine and diltiazem in human myocardium. The effect on force development and intracellular Ca^{2+}-transients was studied simultaneously using the Ca^{2+}-indicator fura-2. To exclude interference of hemodynamic factors such as changes in preload, afterload, or frequency, the calcium antagonists were examined in electrically driven multicellular heart muscle preparations. The effect of calcium antagonists on the vascular tone of precontracted ($PGF_{2\alpha}$) human coronary artery rings was studied as well.

Methods

Patients

Myocardium from terminally failing human hearts (New York Heart Association functional class IV, NYHA IV) was obtained from patients after cardiectomy during cardiac transplantation because of dilated cardiomyopathy (24 patients, 45 ± 4 years, range: 30 – 62). Patients gave written informed consent. The study was approved by the local Ethics Committee. The pretreatment of the patients consisted of ACE-inhibitors, diuretics and nitrates. Right atrial tissue was taken from patients undergoing aortocoronary bypass operation ($n = 31$, age: 68.5 ± 6.0 years, range: 42 – 79 years) without clinical signs of cardiac failure as measured by heart catheterization (normal ejection fraction, enddiastolic volume and stroke volume) and by echocardiography. None of the patients had received Ca^{2+}-channel antagonists or Ca^{2+}-channel agonists within 7 days of surgery, nor β-adrenoceptor agonists 48 h before surgery. Drugs used for general anaesthesia were flunitrazepam, fentanyl and pancuronium bromide with isofluran. The tissue was delivered within 10 min into the laboratory in ice-cold preaerated Bretschneider solution of the following composition (in mmol l^{-1}) NaCl 15, KCl 10, $MgCl_2$ 4, histidine 180, tryptophane 2, mannitol 30 and potassium dihydrogen oxoglutarate 1.

Contraction experiments

The experiments were performed on isolated electrically driven (1 Hz) muscle preparations (left ventricular papillary muscle strips or right atrial trabeculae). Muscle strips of uniform size with muscle fibres running parallel to the length of the strips (diameter < 0.1 mm, length 8 – 10 mm) were dissected in aerated bathing solution (for composition see below) at room temperature. Connective tissue was carefully trimmed away. The preparations were attached to a bipolar platinum stimulating electrode and suspended individually in 75 ml glass tissue chambers for the recording of isometric contractions. The bathing solution used was a modified Tyrode's solution containing (in mmol l^{-1}) NaCl 119.8, KCl 5.4, $MgCl_2$ 1.05, $CaCl_2$ 1.8, $NaHCO_3$ 22.6, $NaHPO_4$ 0.42, glucose 5.05, ascorbic acid 0.28, disodium EDTA 0.05, 37°C, pH 7.4. It was gassed continuously with 95% O_2 and 5% CO_2. The pH of the solution remained constant throughout the experiments. Muscle strips were attached to two stainless metal pins, one of them was mobile connected to a force transducer (W. Fleck, Mainz, Germany) which was attached to a Gould recorder (Gould Inc., Cleveland, U.S.A.). The preparations were paced electrically at 1 Hz with rectangular pulses of 5 ms duration (Grass stimulator SD 9, Qunicy, U.S.A.). The voltage was 20% above threshold. All preparations were allowed to equilibrate at least 90 min in a drug free bathing solution until complete mechanical stabilization. After 45 min, the bathing solution was changed. Concentration-dependent mechanical effects (i.e. force of contraction) of mibefradil, diltiazem and nifedipine were obtained. Control experiments were performed in Tyrode's solution with identical composition. Agents were applied cumulatively to the organ bath. Each muscle was used only once to record a concentration-response curve. Experiments were carried out according to Schwinger *et al*. (1996).

Simultaneous measurement of the intracellular Ca^{2+}-transient and force development

Fura-2 loading Intracellular Ca^{2+} was measured by the fluorescence indicator fura-2 (Grynkiewicz *et al*., 1985). To facilitate cell loading fura-2 was used as acetoxymethyl (AM) ester. These AM esters passively cross the plasma membrane and once inside the cell are cleaved to cell-impermeant products by intracellular esterases (Grynkiewicz *et al*., 1985). For the initial control measurement of force of contraction one end of the muscle strip was clamped at a muscle holder and the other end was attached to a force transducer (SI, Heidelberg, Germany). The muscle fibres were superfused with an oxygenated (95% O_2, 5% CO_2) Tyrode's solution (in mmol l^{-1}) NaCl 119.8, KCl 5.4, $MgCl_2$ 1.05, $CaCl_2$ 0.9, $NaHCO_3$ 22.6, $NaHPO_4$ 0.42, glucose 5.05, ascorbic acid 0.28, disodium EDTA 0.05, 37°C, pH 7.40). The muscles were stretched until the optimal force was generated and were stimulated by a pulse generator (Föhr Medical Instruments GmbH, Egelsbach, Germany) with a square wave pulse (field stimulation) of 10 ms duration (voltage: 10% above threshold voltage, frequency: 1 Hz). Muscle strips with an adequate mechanical performance were incubated for 4 h in darkness to avoid photobleaching of the dye in an oxygenated (95% O_2, 5% CO_2) Ringer-solution (in mmol l^{-1} NaCl 147, KCl 4, $CaCl_2$ 2.2) at 22°C, pH 7.4, containing 5 μmol l^{-1} of fura-2

AM. Preparations were performed as described by Brixius *et al*. (1997).

Ca^{2+}- and force-measurement After fura-2 loading, the muscles were rinsed with oxygenated Tyrode's solution for 15 min. Afterwards the muscle strips were fixed at the both ends between the muscle holder and the force transducer. The force transducer was connected by an AD-converter to a personal computer. For on-line data analysis a special software was used (SI, Heidelberg, Germany).

Fura-2 fluorescence was measured using a dual wavelength fluorometer equipped with an inverted microscope (SI, Heidelberg, Germany). Light was emitted through a mercury arc-lamp (USH – 102DH, Ushio, Tokyo, Japan). A rotating filter wheel allowed alternating excitation at wavelengths of either 340 nm (for the Ca^{2+} fura-2 complex) or 380 nm (Ca^{2+} free fura-2) with a frequency of alteration of 125 Hz. The emitted fluorescence light resulting from the excitation with one of these wavelengths was recorded at 510 nm and sorted in the respective channels of the photomultiplier. Ca^{2+}-transients were digitized and the fluorescence ratio ($R_{340/380}$) was stored in a personal computer. There was a shutter placed between the Hg-arc lamp and the filter wheel to avoid photobleaching of fura-2 by continuous exposure to the excitation light. The shutter could be manually opened by measurements and closed respectively. Thus, the trabeculae were only confronted with the excitation light at definite time points during the experiments. The force of contraction was continuously recorded by an oscilloscope. Control experiments were performed to measure fura-2 fluorescence under the same experimental conditions in muscle strips from the same heart samples without drug addition. Fura-2 fluorescence did not change. Experiments were performed as described by Brixius *et al*. (1997).

Isolated human coronary artery rings

Studies were performed in isolated vascular rings of epicardial coronary vessels. Ring segments (3 – 5 mm) from the proximal left anterior descending coronary artery or circumflex artery were studied after cleaning the arteries from adherent fat and connective tissue. Attention and care were given to maintaining and not disrupting the vascular endothelium. The time from cardiectomy to placement in the organ bath, containing Tyrode's solution (for composition see above) aerated with 95% O_2 and 5% CO_2 was less than 25 min. Human coronary rings were attached to two metal pins. Isometric force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, Germany) connected to a Gould recorder (Gould Inc, U.S.A.). The rings were allowed to equilibrate for at least 90 min until complete mechanical stabilization. The bathing solution was changed at intervals of 20 min. Two grams of resting tension were applied to each coronary segment. At the beginning of the study, the coronary artery rings were placed at their optimal length for tension development. This was accomplished by gradually increasing the resting tension until the reaction to 100 mmol l^{-1} of KCl was optimized. The coronary ring segments were left at that tension throughout the pharmacological testing. To study the vasodilatory actions of Ca^{2+}-channel antagonists, the coronary artery rings were precontracted by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 0.3 μmol l^{-1}). The maximal relaxing potency of the coronary artery rings was studied by the addition of papaverine (100 μmol l^{-1}). The maximal vasodilating effects of the Ca^{2+}-antagonists were compared to the relaxation measured in the presence of 100 μmol l^{-1} papaverine, i.e. the papaverine-induced vasodilation was set to 100%. Concentration-response curves (0.001 – 100 μmol l^{-1}) were recorded after cumulative addition of mibefradile, diltiazem or nifedipine, each being added when the maximal effect had been produced by the previous concentration. The experiments were performed as described by Ginsburg *et al*. (1983) and Schwinger *et al*. (1996).

Materials

Mibefradil (Ro 40-5967) was obtained from Hoffman-La Roche AG (Basel, Switzerland), nifedipine from Bayer AG (Leverkusen, Germany) and diltiazem from Gödecke (Freiburg, Germany). Nifedipine was prepared as a stock solution of 10 mmol l^{-1} in 100% dimethylsulfoxide (DMSO). All other chemicals were of analytical grade or the best grade commercially available. Fura-2 AM was obtained from Molecular Probes (Eugene, Oregon, U.S.A.). A stock solution of fura-2 AM (10 mmol l^{-1}) was dissolved in 100% DMSO and stored at -20°C. The final concentration of DMSO in the organ bath never exceeded 0.05%.

Statistical analysis

Results are presented as mean $\pm$ s.e.m. Statistical significance was determined by Student's *t*-test or one way analysis of variance (ANOVA). The level of statistical significance was set at a probability of 0.05. The vasodilating effects of mibefradil, diltiazem and nifedipine were compared by the concentration necessary to reduce vascular tension to 50% of the papaverine (100 μmol l^{-1})-induced relaxation (100%). The contractile effects of the three Ca^{2+}-channel antagonists were compared by the concentration at which the basal force of contraction was reduced by 25%. These values are given as mean and 95% confidence interval. The concentration required to decrease basal force of contraction by 25% was determined by the computer program 'GraphPad Prism' (GraphPad Prism, San Diego, California, U.S.A.) for each experiment.

Results

Effect of force development

Human atrial myocardium As atrial human myocardium is more sensitive to alterations of the intracellular Ca^{2+}-concentration (Schwinger *et al*., 1990b), the negative inotropic effect of the Ca^{2+}-channel antagonists nifedipine (NIF, $n=8$ from four hearts), diltiazem (DIL, $n=9$ from six hearts) and mibefradil (MIB, $n=7$ from four hearts) was first studied in isolated right auricular trabeculae. The atrial tissue was obtained from patients undergoing aortocoronary bypass operation without clinical signs of cardiac failure. Basal force of contraction (FOC) measured at an extracellular Ca^{2+}-concentration of 1.8 mmol l^{-1} and a stimulation frequency of 1 Hz was similar in all three groups (NIF: 2.9 $\pm$ 0.5 mN, DIL: 2.8 $\pm$ 0.5 mN, MIB: 3.7 $\pm$ 0.7 mN). NIF, DIL and MIB concentration-dependently (0.0001 – 10 μmol l^{-1}) reduced basal FOC (Figure 1). At the highest concentration used (10 μmol l^{-1}), NIF and DIL decreased FOC to 6.5 $\pm$ 0.9% and 8.3 $\pm$ 0.9% of control, respectively. MIB (10 μmol l^{-1}) diminished FOC to only 52.2 $\pm$ 6.1% of its basal value. The negative inotropic potency of the three Ca^{2+}-antagonists was measured by the concentration needed for 25% decrease of basal FOC. The following rank order was obtained NIF

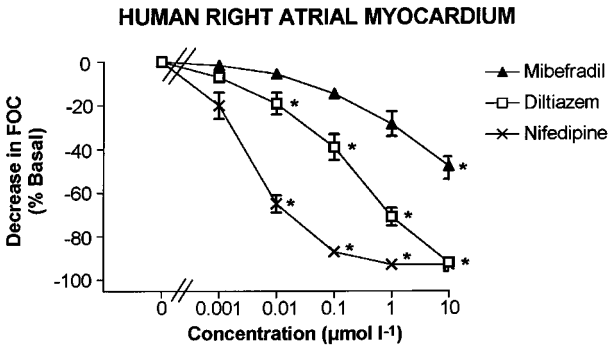

Figure 1 Effect of mibefradil, diltiazem and nifedipine on right atrial human myocardium. Ordinate: Decrease in force of contraction (FOC) as percentage of the basal value; Abscissa: Concentration in μmol l^{-1} *$P<0.05$ versus basal value.

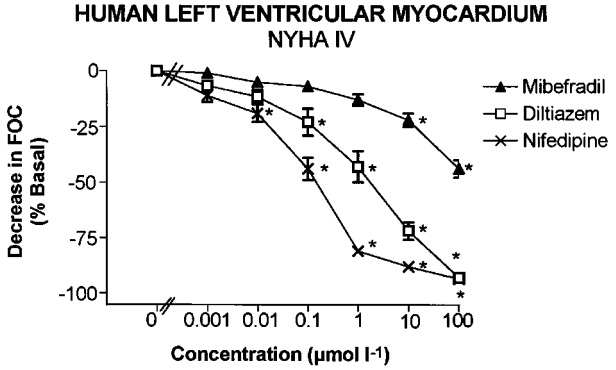

Figure 2 Negative inotropic response of mibefradil, diltiazem and nifedipine in left ventricular human myocardium. Ordinate: Decrease in force of contraction (FOC) as percentage of the basal value; Abscissa: Concentration in μmol l^{-1} *$P<0.05$ versus basal value.

(0.0022 μmol l^{-1}, confidence interval (CI): $0.0005-0.0049$ μmol l^{-1}) > DIL (0.031 μmol l^{-1}, CI: $0.011-0.73$ μmol l^{-1}) > MIB (0.80 μmol l^{-1}, CI: $0.19-1.42$ μmol l^{-1}), i.e. 1 : 14 : 364. Thus, the negative inotropic efficacy and potency of MIB was significantly smaller compared to DIL and NIF in human right atrial tissue.

Human left ventricular myocardium The negative inotropic effect of the three Ca^{2+}-channel antagonists ($0.001-100$ μmol l^{-1}) was also studied in isolated electrically driven left ventricular papillary muscle strips of failing (dilated cardiomyopathy) human myocardium. Under control conditions, FOC did not change during the time needed for the experiment. Basal FOC as measured before the onset of the experiments was similar in the NIF- ($n=9$ from four hearts, 2.5 ± 0.2 mN), DIL- ($n=7$ from three hearts, 2.1 ± 0.2 mN) and MIB-group ($n=15$ from six hearts, 2.5 ± 0.6 mN). Figure 2 summarizes the results. NIF and DIL reduced force of contraction almost completely (100 μmol l^{-1}, NIF: 6.5 ± 1.4% of basal FOC, DIL: 7.1 ± 1.2% of basal FOC), whereas MIB, even at the highest concentration used (100 μmol l^{-1}), only reduced FOC to 55.1 ± 4.0% of the initial FOC. Thus, the rank order of negative inotropic efficacy of the tested Ca^{2+}-antagonists was identical in right atrial and left ventricular myocardium, i.e. NIF > DIL > MIB.

The negative inotropic effect of NIF was significant at concentrations higher than 0.001 μmol l^{-1}, that of DIL at concentrations higher than 0.01 μmol l^{-1}, and MIB significantly reduced FOC at concentrations higher than 1 μmol l^{-1} (Figure 2). Evaluating the concentration needed to achieve 25% reduction of the basal force, a similar rank order of negative inotropic potency as calculated for atrial tissue was found: NIF (0.0095 μmol l^{-1} CI: $0.002-0.04$ μmol l^{-1}) > DIL (0.041 μmol l^{-1} CI: $0.005-0.35$ μmol l^{-1}) > MIB (9.47 μmol l^{-1} CI $1.37-65.2$ μmol l^{-1}), i.e. 1 : 4 : 997. However, in accordance to Schwinger *et al*., 1990b, all three Ca^{2+}-channel antagonists were more potent in the right atrium than in the left ventricle of human hearts to reduce FOC.

Effect on intracellular Ca^{2+}-transient

The influence of NIF ($n=4$ from four hearts), DIL ($n=5$ from five hearts) and MIB ($n=8$ from eight hearts) on the intracellular Ca^{2+}-transient and force of contraction was simultaneously studied in right atrial muscle strips by the fura 2-ratio method (Grynkiewicz *et al*., 1985). Figure 3 shows a representative original tracing of the influence of MIB on the intracellular Ca^{2+}-transient and on FOC. At a concentration

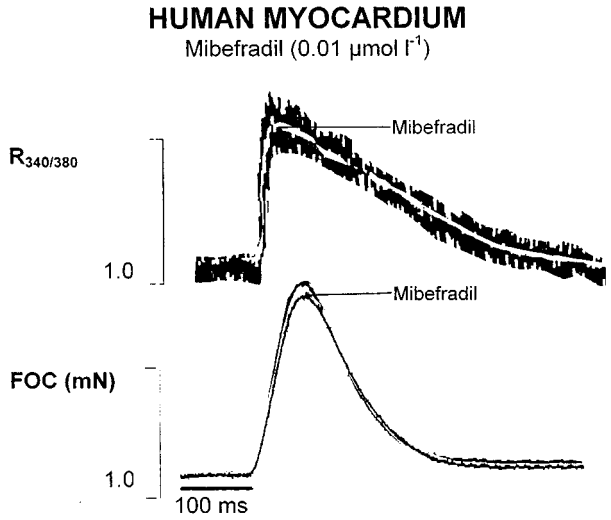

Figure 3 Original tracing of the simultaneous measurement of the intracellular Ca^{2+}-transient and the isometric force of contraction after application of mibefradil (0.01 μmol l^{-1}). FOC: Force of contraction; $R_{340/380}$: Ratio of the intracellular Ca^{2+}-transient measured by the fluorescence indicator fura 2 at 340 nm (fura-2 Ca^{2+}-complex) and 380 nm (Ca^{2+}-free fura-2 complex).

of 0.01 μmol l^{-1}, MIB did not change the intracellular Ca^{2+}-transient nor the contractile twitch. Figure 4 summarizes the results obtained for the changes of the intracellular Ca^{2+}-transient after application of increasing concentrations of NIF ($0.001-1$ μmol l^{-1}), DIL ($0.001-1$ μmol l^{-1}) and MIB ($0.01-1$ μmol l^{-1}). The 340/380 ratios of the fura-2 signal measured before drug application were similar in all three groups investigated (NIF: 0.92 ± 0.15, DIL: 1.07 ± 0.16, MIB: 0.94 ± 0.15). NIF significantly decreased the intracellular Ca^{2+}-transient at all concentrations studied. DIL significantly diminished the fura-2 ratio at concentrations at and above 0.01 μmol l^{-1}, whereas MIB started to significantly reduce the intracellular Ca^{2+}-transient at concentrations at and above 0.1 μmol l^{-1}. Therefore, the rank order of Ca^{2+}-antagonistic property of the three tested drugs was similar to that of the negative inotropic efficacy, i.e. NIF > DIL > MIB.

Effect on vascular tone

Ca^{2+}-antagonists are used in human heart failure because of their vasodilating properties, which may be due to a reduction

of the intracellular Ca^{2+}-concentration in vascular tissue. To study the effects of MIB ($n=8$ from four hearts) on human coronary blood vessels, the changes in tension development of isolated human coronary artery rings precontracted with $PGF_{2\alpha}$ (0.3 μmol l^{-1}) were studied. Figure 5 shows a typical example of cumulatively applied concentrations of MIB, DIL and NIF on tension of isolated coronary artery rings. The maximum vasodilatory potency of the used Ca^{2+}-antagonists was compared to the vasodilating effect of papaverine (100 μmol l^{-1}, upper part of Figure 5). MIB, DIL and NIF concentration-dependently (0.001 – 100 μmol l^{-1}) reduced the vascular tone of $PGF_{2\alpha}$-precontracted human coronary artery rings. MIB (100 μmol l^{-1}) reduced the vascular tension by 81.7 ± 5.5% of the vasodilatory effect of papaverine (100%). The corresponding values for DIL and NIF were 91.3 ± 0.9% and 96.4 ± 3.7%, respectively.

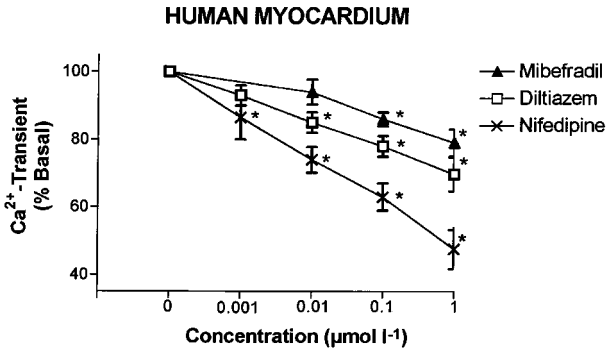

Figure 4 Effect of mibefradil, diltiazem and nifedipine on the intracellular Ca^{2+}-transient. *Ordinate*: Percent decrease of the intracellular Ca^{2+}-transient as measured by the fluorescence indicator fura-2 at 340 nm (fura-2 Ca^{2+}-complex) and 380 nm (Ca^{2+}-free fura-2 complex); *Abscissa*: Concentration in μmol l^{-1} $^*P<0.05$ versus basal value.

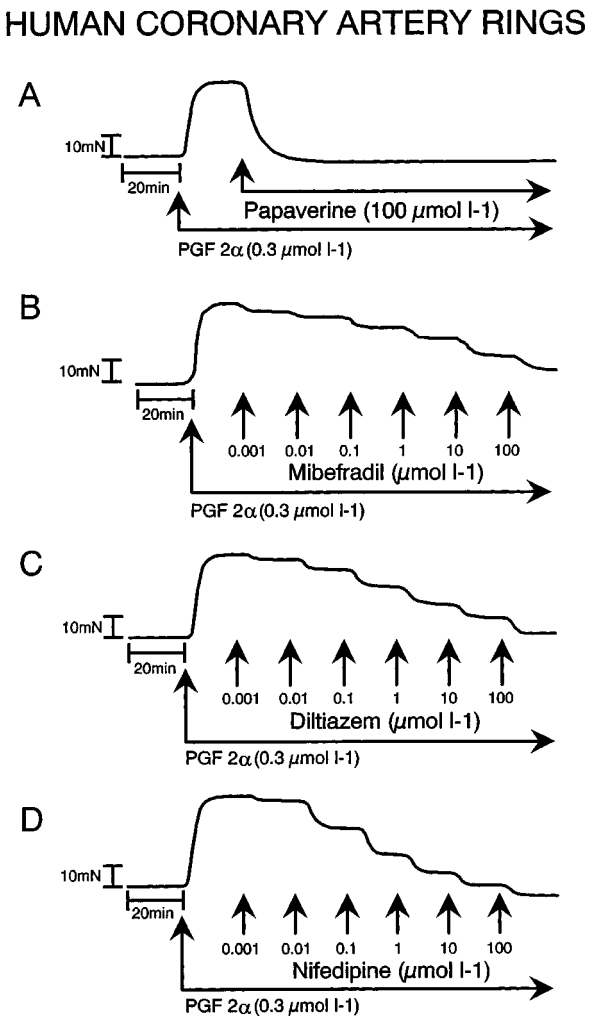

Figure 5 Original tracings of the effect of papaverine (100 μmol l^{-1}), (A) and the concentration-dependent (0.001 – 100 μmol l^{-1}) effects of mibefradil (B), diltiazem (C) and nifedipine (D) on human coronary artery rings precontracted by prostaglandin 2α (PGF 2α). Mibefradil, diltiazem and nifedipine concentration-dependently relaxed human coronary artery rings.

The concentration-dependent effects of MIB, DIL and NIF on tension of precontracted isolated human coronary artery rings are summarized in Figure 6. All Ca^{2+}-channel antagonists exerted a concentration-dependent vasodilatory activity. The rank order for the vasodilatory moiety as judged by the EC_{50}-values was NIF (EC_{50}: 0.02 μmol l^{-1}, confidence interval (CI): 0.05 – 0.2 μmol l^{-1}) > DIL (EC_{50}: 0.13 μmol l^{-1}, Cl: 0.01 – 1.28 μmol l^{-1}) > MIB (EC_{50}: 2.05 μmol l^{-1}, CI: 0.29 – 14.00 μmol l^{-1}), i.e. 1 : 7 : 103. The concentration-response-curve of MIB was significantly shifted rightwards compared to NIF and DIL.

Vasoselectivity

The vasoselectivity of a drug is determined by the ratio of the negative inotropic and the vasodilatory effect. In the present study the concentration necessary for a 25% relaxation of the coronary vessels (100% papaverine) was compared to the concentration needed to reduce basal force of contraction by 25%. In comparison to its vasodilatory effect a 316 fold higher concentration of MIB was necessary to reduce basal FOC by 25%. The corresponding values for NIF and DIL were 1.5 and 1.0, respectively. Thus, MIB has a higher vasoselectivity in human cardiovascular tissue as compared to NIF and DIL.

Discussion

Mibefradil is a novel Ca^{2+}-antagonist, which has a three to four times higher selectivity towards T-type Ca^{2+}-channels in comparison to L-type Ca^{2+}-channels as shown by blockade of divalent ion currents in vascular muscle cells of the azygos vein of 1 to 3-day-old N/nih neonatal rats (Mishra & Hermsmeyer,

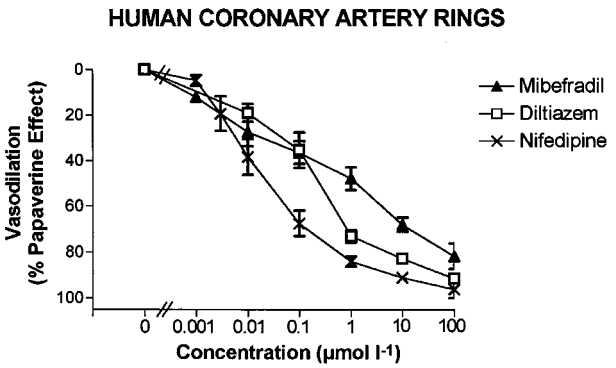

Figure 6 Comparison of the vasodilating effects of the Ca^{2+}-antagonists mibefradil, diltiazem and nifedipine on human coronary artery rings. *Ordinate*: Vasodilation as percent decrease of the maximal value, i.e. the vasodilating effect of 100 μmol l^{-1} papaverine; *Abscissa*: Concentration of mibefradil, diltiazem and nifedipine in μmol l^{-1}.

1994). In Wistar rats with chronic myocardial infarction, mibefradil induced a selective vasodilation of the peripheral vessels without direct negative inotropic effects (Véniant *et al.*, 1991). The regulation of contraction is not only species-dependent, but also relying upon the possible underlying cardiac disease. Therefore, the present study aimed to examine the vascular and inotropic actions of T-type Ca^{2+}-current-inhibition in man. It was demonstrated that mibefradil is an effective vasodilator of human coronary arteries. In concentrations with significant vasodilating actions, mibefradil exerted only minor negative inotropic effects in human myocardium.

Effect on force of contraction

The cardiovascular effects of Ca^{2+}-antagonists are mediated by a decrease of the transmembraneous Ca^{2+}-influx during contraction (Henry, 1980). At a concentration of 0.01 μmol l^{-1} mibefradil had no influence on the intracellular Ca^{2+}-transient and on force of contraction as shown in this study. However, at higher concentrations, mibefradil also reduced the contractile twitch in human right atrial and left ventricular myocardium, as well as the intracellular Ca^{2+}-transient. In accordance, Hensley *et al.* (1997) found that mibefradil even increased the intracellular Ca^{2+}-transient by 16% at concentrations of 0.00001 μmol l^{-1} and 0.0001 μmol l^{-1}, but decreased the intracellular Ca^{2+}-transient by 25% at a concentration of 1 μmol l^{-1} in canine cardiomyocytes. Nifedipine was inhibitory at all concentrations tested (0.00001 – 1 μmol l^{-1}). Furthermore, these results are in line with a study on the hemodynamic and cardiac effects of mibefradil in patients with varying degrees of left ventricular systolic dysfunction which demonstrated that only high plasma levels of mibefradil depressed the maximal first derivative of left ventricular pressure (Rousseau *et al.*, 1996). As T-type Ca^{2+} currents do not seem to make a detectable contribution to the transmembrane Ca^{2+}-influx in human myocardium (Beuckelmann *et al.*, 1991), these negative inotropic actions of mibefradil at very high concentrations may be related to inhibition of L-type Ca^{2+}-channels. Ratner *et al.* (1996) concluded from binding studies on cardiac and aortic membranes of human hearts that mibefradil binds to a site of the L-type Ca^{2+} channel which is different from the verapamil-binding site, but that this site could be allosterically linked to the phenylalkylamine binding site.

The enhanced sensitivity of the atrial tissue towards Ca^{2+}-antagonists may indicate that atrial tissue is more dependent on sarcolemmal Ca^{2+}-fluxes than the left ventricular myocardium (Schwinger *et al.*, 1990b). However, it cannot be excluded that the differences found between atrial tissue from patients undergoing aortocoronary bypass operation and left ventricular myocardium of patients with dilated cardiomyopathy are due to the underlying disease process. Nuss and Houser (1993) investigating T-type Ca^{2+} currents in hypertrophied adult feline left ventricular myocytes showed that T-type Ca^{2+} channels may be reexpressed in adults in association with hypertrophy resulting from slow progressive pressure overload. Sen & Smith (1994) investigated T-type Ca^{2+}-channels in genetically determined cardiomyopathic hamster hearts. They demonstrated that the mean current density of T-type Ca^{2+} channels in cardiomyopathic cells was significantly higher than in normal cells. Yet, these results are in contrast to the present findings: mibefradil is more efficacious in the atrial-healthy compared to the left ventricular-diseased myocardium. In accordance with the present study, Beuckelmann *et al.* (1991) found that T-type Ca^{2+}-currents do not seem to make a detectable contribution to the transmembrane Ca^{2+}-influx in human ventricular myocytes of patients with severe heart failure. In addition, the Ca^{2+}-current of L-type Ca^{2+}-channels measured was significantly increased in atrial (patients undergoing bypass surgery) compared to left ventricular myocardium (patients with dilated cardiomyopathy). Therefore, the present study provides evidence that the differences found between atrial and ventricular myocardium may be due to pathological as well as anatomical differences.

Effect on vasodilation

In the present study the vasodilatory effects of Ca^{2+}-channel antagonists were studied *in vitro* in isolated human coronary artery rings. The vasodilatory potency of mibefradil was smaller compared to that of the Ca^{2+}-antagonists diltiazem and nifedipine. Direct comparable studies investigating the three Ca^{2+}-antagonists on isolated coronary artery rings of humans or animals are still lacking. It has been shown by the Mibefradil Study Group that a once-daily treatment with mibefradil (100 mg/d) is significantly more effective than diltiazem CD (360 mg/d) in lowering diastolic and systolic blood pressure (Bittar, 1997). In another study (Massie *et al.*, 1997), mibefradil (100 mg/d) significantly decreased the sitting diastolic blood pressure (14 ± 7.8 mmHg) compared with diltiazem CD (360 mg/d; 9.5 ± 7.5 mmHg) and nifedipine SR (40 mg/three times a day; 8.1 ± 19.2 mmHg). *In vivo* mibefradil might be more effective than diltiazem in lowering blood pressure because of its higher mean therapeutic active plasma concentration (0.87 μmol l^{-1}, Clozel *et al.*, 1991) compared to diltiazem (0.19 μmol l^{-1}, Henry, 1980) used because of the lack of negative inotropic effect at these concentrations. In addition, it has been shown that chronic treatment with mibefradil potentiated the endothelium-dependent relaxation in the aorta of hypertensive salt sensitive Dahl rats (Boulanger *et al.*, 1994). This might also contribute to the enhanced potency and efficacy of mibefradil. Thus, the minor negative inotropic effect of mibefradil is the prerequisite for its potent vasorelaxing actions *in vivo*.

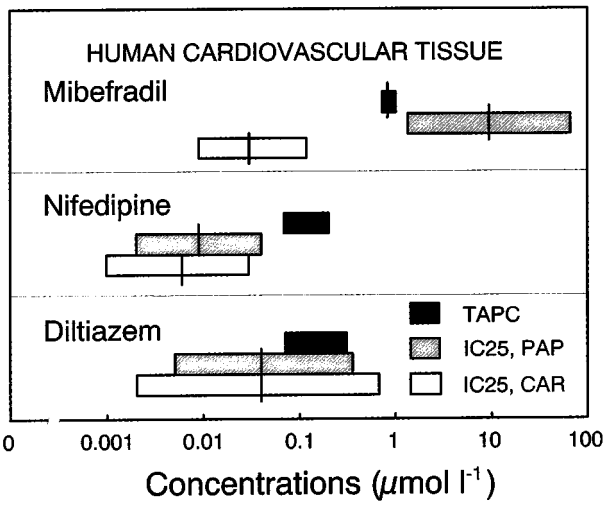

Figure 7 Clinical implications of Ca^{2+}-channel antagonists in the treatment of heart failure patients. TAPC: therapeutic active plasma concentration (diltiazem, nifedipine: Henry (1980); mibefradil: Clozel *et al.* (1991)), IC_{25}: drug concentration required to decrease the developed force of contraction by 25% of the basal force of contraction in multicellular muscle strips as well as in isolated coronary artery rings, PAP: isolated, electrically driven, left ventricular muscle strip, CAR: coronary artery rings.

Vasoselectivity

Based on the relation between therapeutic active plasma concentrations, vasoactive concentrations as well as negative inotropic effective concentrations in the *in vitro* system, a rank order of vasoselectivity can be established (Figure 7). In contrast to the L-type Ca^{2+}-channel antagonists nifedipine and diltiazem, the therapeutic active plasma concentration of mibefradil is very much lower than the concentration at which negative inotropic effects were observed in failing human myocardium. Thus, mibefradil is the only Ca^{2+}-channel antagonist with vasodilatory effects at concentrations significantly lower than these concentrations, which produce negative inotropic effects. This study confirms previous results obtained from animal studies, e.g. normal and failing rat (Clozel *et al*., 1990), rats with chronic myocardial infarction (Véniant *et al*., 1991), guinea pigs (Clozel *et al*., 1991), that mibefradil represents a selective vasodilator of the peripheral vessels with small direct negative inotropic effect.

In conclusion, the negative inotropic effect of mibefradil at high concentrations may be due to a blockade of the sarcolemmal L-type Ca^{2+}-channels. The increased vasoselectivity of mibefradil may be especially advantageous in the treatment of patients with an already diminished left ventricular function. Additional studies are needed on the mode of Ca^{2+}-influx of T-type channels, regional distribution, and whether or not T-type Ca^{2+}-channels may be expressed in a different amount in nonfailing versus diseased myocyte, i.e. hypertrophy, ischemic cardiomyopathy, dilated cardiomyopathy.

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