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Levosimendan improves diastolic and systolic function in failing human myocardium

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Abstract

Ca^{2+}-sensitizers increase myocardial contractility, but may worsen diastolic dysfunction. Levosimendan, through its unique troponin-C interaction, may preserve diastolic function. We investigated the effects of levosimendan (10^{-7}–10^{-5} M) on diastolic and systolic function in multicellular cardiac muscle preparations from end-stage failing human hearts (1 and 2.5 Hz, 37°C, 1.25 mM [Ca^{2+}], pH 7.4). Levosimendan improved systolic function: at 1 Hz, developed force (F_{dev}) increased from 13.84 ± 3.27 to 16.40 ± 3.57 (10^{-7} M, $P < 0.05$), while diastolic force (F_{dia}) decreased from 5.32 ± 0.67 to 4.94 ± 0.61 mN/mm^2 ($P < 0.05$). Under control conditions, the increase in stimulation frequency from 1 to 2.5 Hz resulted in a decrease in F_{dev} of -0.51 ± 1.80 mN/mm^2 (negative force–frequency relationship). Levosimendan improved this relationship: at 10^{-7} M, this change became positive ($+1.81 \pm 2.06$ mN/mm^2, $P < 0.05$). Diastolic function was markedly improved in the presence of levosimendan; the increase in F_{dia} of 1.56 ± 0.42 mN/mm^2 (control) was attenuated to 0.70 ± 0.19 mN/mm^2 ($P < 0.05$). To allow for a more detailed analysis, preparations were sometimes divided into two groups, based on their force–frequency behavior. Twitch timing parameters were accelerated by levosimendan in preparations with a negative force–frequency relationship. Levosimendan improves both systolic and diastolic function in failing human myocardium. Effects are even more pronounced at higher heart rates and under prevailing diastolic dysfunction.

Keywords: Ca^{2+}-sensitizer; Inotropic agent; Hemodynamics; Heart failure; Excitation–contraction coupling; Diastolic dysfunction

1. Introduction

Despite the apparent need for increased inotropic stimulation of the failing human heart to provide sufficient pumping capability, little has been accomplished in establishing a therapy that acts positive inotropically without negatively impacting on long-term outcome. A large number of inotropic strategies target the cyclic-adenosine-monophosphate (cAMP) dependent pathway. These drugs may be less efficient in end-stage failing myocardium, where the importance of this pathway is reduced because of down-regulated β-adrenoceptors, up-regulated inhibitory G protein (G_i), down-regulated stimulatory G-protein (G_s), and reduced cAMP concentrations (Bristow et al., 1982; Böhm et al., 1990; Feldman et al., 1988). In addition, a deleterious effect on energy demand might hamper applicability of drugs that mainly act through the cAMP pathway (Hasenfuss et al., 1996). These effects may contribute to a negative impact on long-term patient survival as has been shown for phosphodiesterase inhibitors and catecholamines (Dies et al., 1986; Packer et al., 1991; Uretsky et al., 1990).

Ca^{2+}-sensitizers are a different class of drugs that might potentially be used to increase the hearts' ability to contract more forcefully. These could overcome the limited availability of Ca^{2+} that has been shown to occur in heart failure because of decreased sarcoplasmic reticulum calcium uptake and depressed systolic calcium transients (Gwathmey et al., 1990; Arai et al., 1993; Hasenfuss et al., 1994). However, in light of the impaired relaxation that often accompanies systolic dysfunction (Grossman, 1990), general enhancement of calcium sensitivity might have

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negative effects; increased sensitivity to calcium during diastole would further hamper relaxation of the heart, worsening diastolic dysfunction (Hajjar et al., 1997).

Levosimendan, a novel Ca^{2+}-sensitizer with phosphodiesterase-III inhibitory properties, is an inotropic agent that improves systolic function (Hasenfuss et al., 1998) and may have a possible clinical applicability in patients with heart failure. Unlike most other Ca^{2+}-sensitizers, levosimendan acts through direct binding with troponin-C thereby increasing the affinity of troponin-C for Ca^{2+} in a Ca^{2+}-dependent manner. A lack of Ca^{2+}-sensitization under low prevailing calcium concentrations (i.e. during diastole) might be of critical importance to prevent a worsening of diastolic dysfunction. The phosphodiesterase-III inhibitory component might exert a positive effect on the rate of relaxation (i.e. shorten relaxation). A prolonged relaxation time has been shown to further impair diastolic function when using other Ca^{2+}-sensitizers including EMD 50733 and 53998, ORG 30029, and CGP 48506 (Hajjar et al., 1997; Higashiyama et al., 1995; Zimmermann et al., 1998; Haikala et al., 1995a). The effect of levosimendan on diastolic properties and cardiac relaxation is incompletely understood.

Accordingly, we investigated the effect of therapeutically relevant concentrations of levosimendan (0.1–10 μM) on diastolic function in end-stage failing human myocardium. In multicellular preparations obtained from end-stage failing hearts, systolic and diastolic function was investigated under baseline conditions and under conditions with prevalent high diastolic Ca^{2+} concentration, which were achieved through a physiological relevant mechanism: an increase in the stimulation frequency from 60 to 150 beats/min. Levosimendan improved systolic function without impairing relaxation, and not only lacked a negative effect on diastole; it even improved relaxation at the higher stimulation frequency. The beneficial effects on systolic and diastolic function resulted in positivation of the negative force–frequency relation of failing human myocardium.

2. Methods

2.1. Muscle preparation

Human multicellular muscle preparations ($n = 27$) were dissected from end-stage failing hearts (ischemic cardiomyopathy, $n = 8$ hearts, or dilated cardiomyopathy $n = 1$ heart) that were obtained from patients undergoing cardiac transplantation. Seven preparations were taken from the left ventricle, 20 from the right ventricle. Patient characteristics (all male) were (average ± S.D.); age 64.7 ± 4.1 years; weight 73.3 ± 11.8 kg; left ventricular ejection fraction 26 ± 9%; pulmonary capillary wedge pressure 24 ± 7 mm Hg; and cardiac index 1.90 ± 0.39 l min^{-1} m^{-2}. Medication (number of patients) included angiotensin converting enzyme inhibitors/angiotensin AT_1 receptor antagonists (9), digitalis (8), diuretics (8), nitrates (6), β-adrenoceptor antagonists (2), calcium channel antagonists (2), and statins (1). Hearts were transported in a modified, ice-cold Krebs–Henseleit (K–H) solution containing (in mM): 120 NaCl, 5.0 KCl, 2.0 $MgSO_4$, 1.2 NaH_2PO_4, 20 $NaHCO_3$, 10 glucose, 0.25 $CaCl_2$, with the addition of 20 mM 2,3-butanedione monoxime as a cardioprotective agent.

Muscle preparations were dissected with the aid of a stereo microscope. Free running trabeculae from the endocardial surface were carefully dissected and dimensions were measured at 40 × magnification (resolving power ~ 10 μm). Preparations were mounted in the experimental set-up in the 2,3-butanedione monoxime-containing K–H solution, which was immediately switched to a K–H solution without 2,3-butanedione monoxime, containing also 0.25 mM $[Ca^{2+}]$. All K–H solutions were kept at equilibrium with 95% O_2/5% CO_2, resulting in a pH of 7.4. Human muscle preparations were discarded when under baseline conditions (37 °C, 1.25 mM $[Ca^{2+}]$, stimulation frequency 1 Hz) developed force (F_{dev}) was < 3 mN/mm^2 ($n = 2$, both left ventricular) or run-down during the experiment exceeded 15%/h ($n = 3$, one left ventricular, two right ventricular). Average dimensions of the preparations included in the study were 0.44 ± 0.05 mm wide, 0.35 ± 0.04 mm thick, and 3.78 ± 0.32 mm long ($n = 22$). To avoid artifacts due to improper oxygenation of preparations thicker than 500 μm, only seven were initially taken from the left ventricle. However, the low number of suitable left ventricular preparations (< 500 μm) included in the study ($n = 4$) precluded differential analysis between right and left ventricular preparations.

2.2. Mechanical measurements

The human muscle preparations were mounted using two blocks of ventricular or valvular tissue in the experimental set-up between a basket-shaped extension of a force transducer and a hook connected to a micro-displacement device (Janssen et al., 1999). Following mounting of the muscles, superfusion with K–H (at 37°C) was started and the $[Ca^{2+}]$ was raised from 0.25 to 1.25 mM in steps of 0.25 mM every 2–5 min. When a $[Ca^{2+}]$ of 1.25 mM was reached, stimulation was started through 5-ms asymmetric pulses at 20% above threshold voltage (typically 2–4 V) at 1.0 Hz. At 1.25 mM $[Ca^{2+}]$, the muscle was carefully stretched in several steps until diastolic force (F_{dia}) was about 30% of active F_{dev} or was about 5 mN/mm^2. This preload was chosen because (i) this reflects a sarcomere length of around the maximal end-diastolic length in situ, (ii) evaluation of F_{dia} is not hampered by large amounts stretch-relaxation that occur when such human preparations are stretched to the length where F_{dev} is optimal (i.e., L_{max}), and (iii) using this method, no

off-set differences are expected between preparations with a negative and a positive force–frequency behavior. Data regarding initial F_{dev} (at 5 mN/mm^2 preload) learned that both groups had similar twitch F_{dev}, indicating Frank–Starling curves were similar between the groups, at least at the level of stretch in the preparations in this study.

The muscles were left contracting under these conditions for an additional hour to equilibrate. The following mechanical parameters were measured: active F_{dev} (mN/mm^2), F_{dia} (mN/mm^2), time from stimulation to peak tension (in ms), and times from peak tension to 50% relaxation ($RT_{50\%}$, in ms), and to 90% relaxation ($RT_{90\%}$). Relation times $RT_{50\%}$, and $RT_{90\%}$were calculated as time-point where the relaxation reaches 50% (or 10%) of the amplitude of that twitch, calculated from time to peak tension. In addition, we investigate the maximal negative derivative of force development, normalized to developed tension ($-dF/dt/F_{max}$, in s^{-1}) as a second method to assess relaxation behavior, although this parameter only reflects one single time point of the entire relaxation process.

2.3. Experimental protocol

After baseline data were collected at 1 Hz (reflecting a resting heart rate), stimulation frequency was increased to 2.5 Hz (reflecting a "stressed" or "exercised" heart rate). Under these conditions, diastolic relaxation is incomplete in failing human myocardium, resulting in increased F_{dia}. The F_{dia} is under these conditions not only determined by the passive properties of the tissue, but also by active, force generating cross-bridges. This allowed us to study diastolic dysfunction and allowed for measurement of the impact of levosimendan on diastolic function. After measurements have been taken at 2.5 Hz stimulation frequency was reduced to 1 Hz. Thereafter, measurements were repeated to determine run-down of the preparation by comparing to the "pre-2.5 Hz" baseline. Using only two frequencies minimized this run-down; our experience has learned that performing extensive force–frequency measurements of three or more frequencies rapidly accelerates run-down of these human preparation. After these baseline measurements, levosimendan was added to the solution to a final concentration of 10^{-7} M. Levosimendan, from 0.1-M stock dissolved in dimethylsulfoxide, was diluted with K–H buffer (levosimendan was a gift of Orion Pharma, Finland). Thus, dimethylsulfoxide was limited to 0.01 vol% at the highest concentration used in our studies. A pilot-series of experiments showed that this 0.01 vol% dimethylsulfoxide did not influence any of the parameters investigated. The protocol was repeated and measurements of twitch contractions were collected at 1, 2.5, and again at 1 Hz stimulation frequency. Consecutively, this protocol was repeated in presence of 10^{-6} and thereafter at 10^{-5} M levosimendan, always in the same order.

2.4. Data analysis and statistics

Data were both collected and analyzed off-line with custom-designed data-acquisition programs (LabView, National Instruments). The programs contained an on-line analysis mode to quantify contractile parameters during the experiment. Statistical significance was determined by Students' *t*-test for paired or unpaired data where applicable. When multiple concentrations were compared in two groups, repeated measures analysis of variance (ANOVA) was used, using a two-factorial design (concentration of levosimendan and behavior of force–frequency relationship). Data are expressed as means ± S.E.M. unless stated otherwise. Two-tailed values of $P < 0.05$ were accepted as significant.

3. Results

3.1. Baseline contractility

In total, 22 muscles met the pre-set conditions and were included in the analysis. At 1.25 mM [Ca^{2+}], 37°C and a stimulation frequency of 1 Hz, maximal active F_{dev} amounted to 15.69 ± 3.61 mN/mm^2, while F_{dia} was 4.83 ± 0.64 mN/mm^2. Upon increase of the stimulation frequency to 2.5 Hz, F_{dia} increased significantly to 6.40 ± 0.78 mN/mm^2 ($P < 0.05$) and a non-significant change in F_{dev} was observed and amounted to 15.32 ± 4.20 mN/mm^2.

Alterations of force–frequency behavior in failing human myocardium have been observed in numerous studies, but is some end-stage failing hearts the positive force–frequency behavior is preserved (Mulieri et al., 1982; Janssen et al., 1999). Therefore, we divided all preparations into two groups, one that displayed an increase in F_{dev} upon increase in stimulation frequency (positive force–frequency relationship), and one that displays a decrease in F_{dev} upon increase in stimulation frequency (negative force–frequency relationship), and re-examined baseline contractility in the two groups separately. Examples of the baseline protocol from both groups are given in Fig. 1. In the preparations with a positive force–frequency relationship, baseline F_{dev} at 1 Hz (16.76 ± 6.64 mN/mm^2) was not different from the group with the negative force–frequency relationship (14.80 ± 4.21 mN/mm^2), nor was F_{dia} different (4.29 ± 0.76 vs. 5.27 ± 1.04 mN/mm^2, positive vs. negative force–frequency relationship). Also, dimensions of the preparations were not significantly different between the groups. Upon increase in stimulation frequency to 2.5 Hz, the positive force–frequency relationship group increased F_{dev} to 23.53 ± 8.25 mN/mm^2 ($P < 0.05$) while F_{dia} increased to 5.33 ± 0.96 mN/mm^2 ($P < 0.05$). In the negative force–frequency relationship group F_{dev} decreased to 9.32 ± 3.31 mN/mm^2

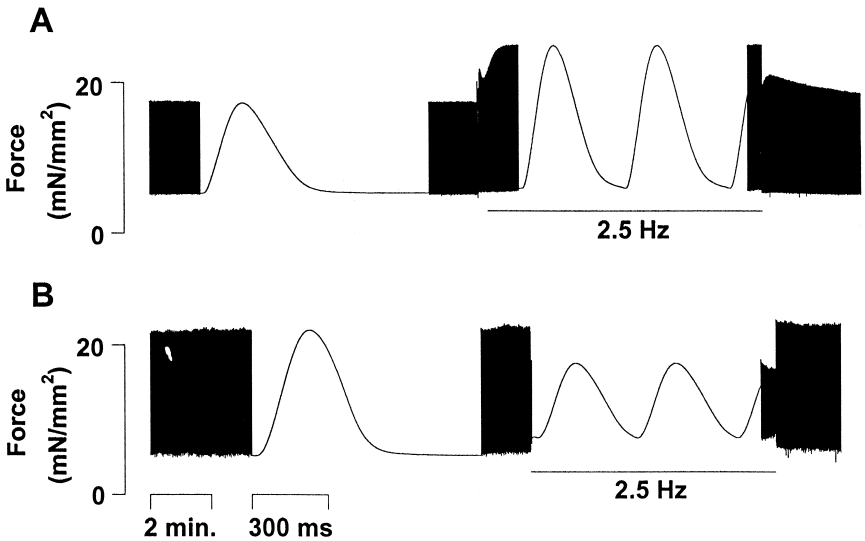

Fig. 1. Effect of increase in stimulation frequency (from 1 to 2.5 Hz) under baseline contractile conditions (37°C, pH 7.4, [Ca^{2+}] 1.25 mM). (A) Example of a preparation with a positive force–frequency relationship. Diastolic tension increases, and an increase in stimulation frequency results in an increase in developed tension. (B) In a subset of preparations, an increase in stimulation frequency results in a decrease in developed tension, while diastolic tension increases (negative force–frequency behavior).

($P < 0.05$), while F_{dia} rose to 7.29 ± 1.20 mN/mm^2 ($P < 0.05$). Upon return to 1 Hz, in both groups F_{dev} was slightly depressed and F_{dia} increased compared to baseline before the 2.5 Hz stimulation. No correlation was found between changes in diastolic tension, direction (positive or negative) of force–frequency behavior, and muscle dimension. Repetitive measurements of baseline contraction allowed for correction of this "run-down" of the preparation. Changes in F_{dev} were calculated from baseline before intervention, and could be corrected for rundown by comparing baseline under the same conditions earlier in the experiment, when necessary. Data were equalized by correcting F_{dev} by this factor. Baseline values for comparison with levosimendan were taken just prior to application of levosimendan.

Baseline twitch timing parameters are given in Table 1. There was a significant difference between the groups even under control conditions. In preparations with a negative force–frequency relationship both time to peak tension and relaxation times were significantly longer. This "impaired relaxation" is the major contributor to the negative force–frequency relationship in these preparations. Although the maximal normalized rate of force decline ($-dF/dt/F_{max}$) was higher also in preparations with a positive force–frequency relationship, it did not quite reach statistical significance ($P = 0.08$).

3.2. Influence of levosimendan on baseline contractility

After application of levosimendan, F_{dev} rose over the time-course of 5–10 min before stabilizing. This was observed in all preparations, and for all concentrations. Addition of 10^{-7} M levosimendan increased F_{dev} in all 22 preparations (from 13.84 ± 3.27 to 16.40 ± 3.57 mN/mm^2, $P < 0.05$), and F_{dia} decreased from 5.32 ± 0.67 to 4.94 ± 0.61 ($P < 0.05$), as can be seen in Fig. 2A. In the group with a positive force–frequency relationship, the increase in F_{dev} was larger (34% on average) compared to

Table 1
Twitch timing parameters at 1 and 2.5 Hz stimulation frequency and before and after addition of levosimendan

Group	TTP (ms)	$RT_{50\%}$ (ms)	$RT_{90\%}$ (ms)	$-dF/dt/F_{max}$ (s^{-1})
All (n = 22)				
Control (1 Hz)	207 ± 7	124 ± 5	258 ± 19	6.05 ± 0.34
Control (2.5 Hz)	171 ± 6[a]	107 ± 4[a]	195 ± 9[a]	6.99 ± 0.36[a]
Lev. 10^{-7} M (1 Hz)	206 ± 6	121 ± 4	242 ± 14	6.42 ± 0.37[a]
Lev. 10^{-6} M (1 Hz)	199 ± 6	121 ± 5	235 ± 13	6.59 ± 0.39[a]
Lev. 10^{-5} M (1 Hz)	188 ± 7	121 ± 6	234 ± 14	6.48 ± 0.43[a]
Positive FFR (n = 10)				
Control (1 Hz)	191 ± 7[b]	113 ± 5[b]	218 ± 15[b]	6.32 ± 0.55
Control (2.5 Hz)	168 ± 9[a]	105 ± 7[a]	189 ± 16[a]	7.34 ± 0.69[a]
Lev. 10^{-7} M (1 Hz)	198 ± 7	115 ± 7	219 ± 19	6.59 ± 0.62
Lev. 10^{-6} M (1 Hz)	193 ± 8	115 ± 7	218 ± 19	6.73 ± 0.65
Lev. 10^{-5} M (1 Hz)	179 ± 10[a]	110 ± 7[b]	208 ± 18	6.85 ± 0.62[a]
Negative FFR (n = 12)				
Control (1 Hz)	220 ± 11[b]	134 ± 8[b]	292 ± 31[b]	5.82 ± 0.46
Control (2.5 Hz)	173 ± 8[a]	109 ± 6[a]	200 ± 9[a]	6.74 ± 0.41[a]
Lev. 10^{-7} M (1 Hz)	212 ± 10[a]	126 ± 6[a]	262 ± 21[a]	6.27 ± 0.50[a]
Lev. 10^{-6} M (1 Hz)	204 ± 10[a]	127 ± 6[a]	249 ± 18[a]	6.48 ± 0.52[a]
Lev. 10^{-5} M (1 Hz)	194 ± 11[a]	128 ± 8[b]	253 ± 19[a]	6.15 ± 0.69

TTP: time from stimulation to peak force development; $RT_{50\%}$ ($RT_{90\%}$): time from peak force to 50% (90%) relaxation; $-dF/dt/F_{max}$: maximal normalized negative derivative of force. Lev: Levosimendan.

[a] Significant difference compared with the control at 1 Hz in the same group ($P < 0.05$).

[b] Significant difference between the group of muscles with a positive and a negative force–frequency relationship ($P < 0.05$).

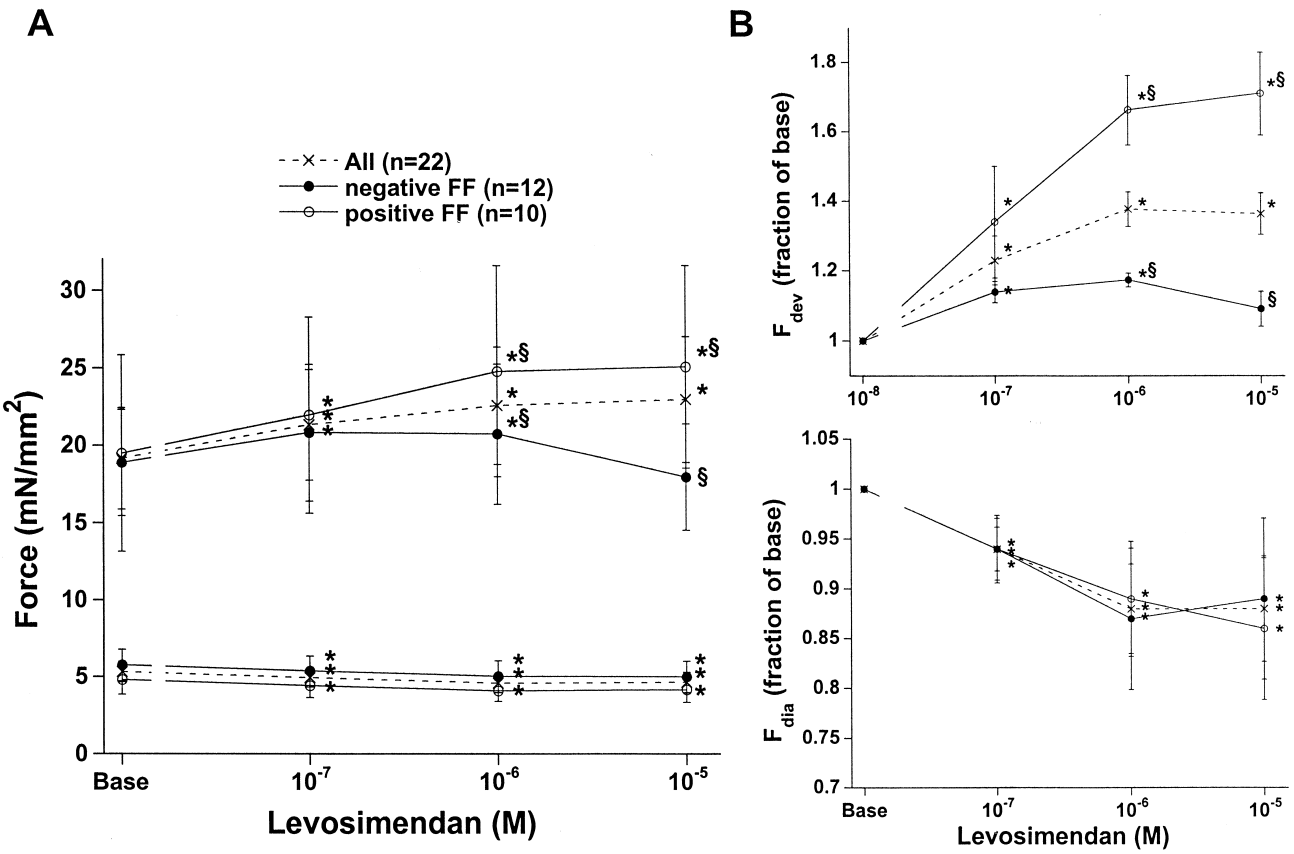

Fig. 2. Effects of levosimendan on contractile parameters. (A) Systolic (top) and diastolic (bottom) force development during baseline conditions, and at increasing concentrations of levosimendan. The response on developed force was more pronounced in preparations with an initial positive force–frequency behavior. (B) Because of the considerable data spread, the same data are expressed as fraction of initial developed (top) or diastolic force (bottom). The * denotes a significant difference compared to baseline ($P < 0.05$). The § denotes a significant difference between preparations with a positive and a negative force–frequency behavior ($P < 0.05$).

the group of preparations with a negative force–frequency relationship (13% on average) (Fig. 2B). At the highest concentration of levosimendan (10^{-5} M) a slight decrease in F_{dev} was observed in preparations exhibiting a negative force–frequency relationship. Effects of levosimendan on F_{dia} are also given in Fig. 2, panels A and C. Clearly, levosimendan did not impair diastolic function, as would have been expected and has been observed with classic Ca^{2+}-sensitizers, but improved diastolic function (decrease in F_{dia}). Regarding F_{dia}, no differences were observed between the preparations with a negative and a positive force–frequency relationship. The effects of levosimendan on steady state twitch timing parameters are given in Table 1. Analysis between the groups indicated that at all concentrations of levosimendan, in preparations with a negative force–frequency relationship twitch duration parameters time to peak tension, time from peak tension to 50% and to 90% relaxation decreased (indicating less impaired, hence improved, relaxation) compared to baseline conditions, whereas in preparations with a positive force–frequency relationship a decrease of time to peak tension and time from peak tension to 90% relaxation was only significant for 10^{-5} M levosimendan. Maximal rate of force decline was significantly faster in preparations with a negative force frequency relationship at 10^{-7} and 10^{-6} M levosimendan, and significantly faster in preparations with a positive force frequency relationship at 10^{-5} M levosimendan. Thus, mainly in the preparations with a negative force–frequency relationship and impaired baseline relaxation, levosimendan significantly improved relaxation. Differences in relaxation times that existed between these groups prior to administration of levosimendan were much smaller and were not statistically significant after application of levosimendan (10^{-7} M).

3.3. Levosimendan and force–frequency relationship

In presence of 10^{-7}, 10^{-6}, and 10^{-5} M levosimendan the stimulation frequency was increased to 2.5 Hz to investigate levosimendans' effect on the force–frequency relationship and active F_{dia}. Levosimendan had pronounced effects on the force–frequency relationship (Fig. 3A). In absence of levosimendan the average ($n = 22$) change in F_{dev} from 1 to 2.5 Hz was -0.51 ± 1.80

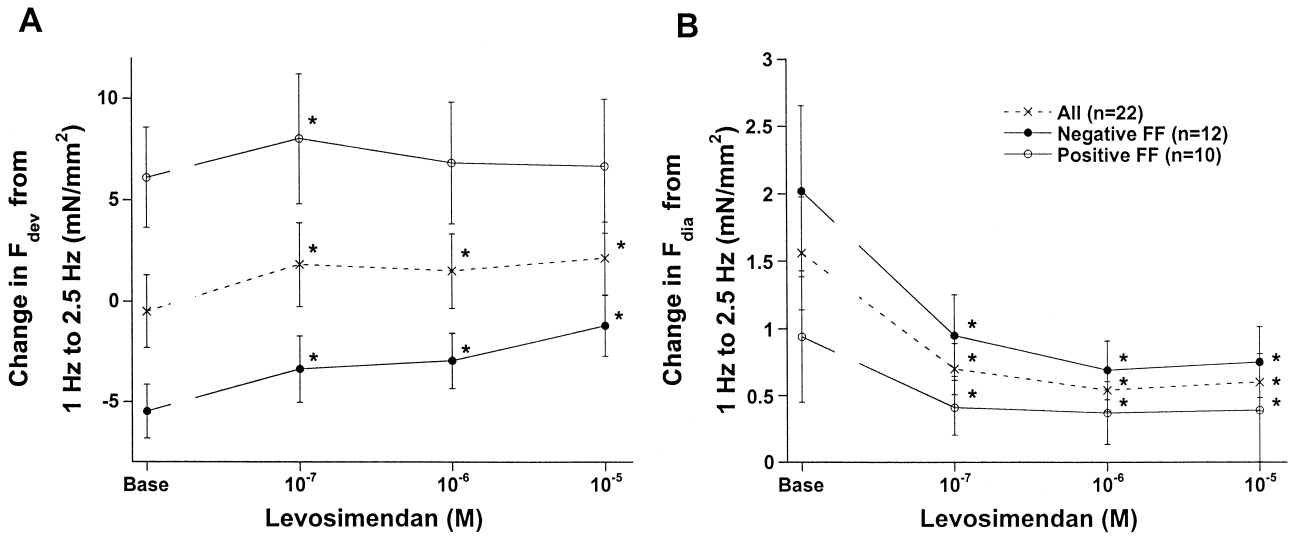

Fig. 3. Change of contractile parameters under elevated diastolic stress, evoked by an increase in stimulation frequency from 1 to 2.5 Hz. (A) Changes in developed force after switching stimulation frequency from 1 to 2.5 Hz. Preparations with an initially negative force–frequency (closed circles) became less negative. Preparations with a positive force–frequency (open circles) became more positive at 10^{-7} M levosimendan and remained unaltered at higher concentrations. (B) Pronounced improvement in diastolic function was observed in all preparations. In presence of levosimendan, diastolic tension was significantly lower compared to baseline conditions. The * denotes a significant change compared to baseline conditions ($P < 0.05$).

mN/mm^2 (negative force–frequency relationship), while in presence of levosimendan (10^{-7} M) it was $+1.81 \pm 2.06$ mN/mm^2 (positive force–frequency relationship). This effect was even more pronounced at higher concentrations of levosimendan ($+2.13 \pm 1.81$ mN/mm^2). Discriminating between baseline force–frequency relationship revealed that preparations with an initial negative force–frequency relationship became less negative in presence of levosimendan, from -5.84 ± 1.34 (control) to -3.37 ± 1.65 at 10^{-7} M ($P < 0.05$) and to -1.19 ± 1.52 mN/mm^2 at 10^{-5} M ($P < 0.05$). Preparations with an initial positive force–frequency relationship became more positive in presence of levosimendan, from $+6.10 \pm 2.47$ (control) to $+8.03 \pm 3.21$ at 10^{-7} M ($P < 0.05$) and to $+6.69 \pm 3.31$ mN/mm^2 at 10^{-5} M ($P < 0.05$ vs. control).

The improvement in the force–frequency relationship is also reflected in the behavior of the F_{dia} (Fig. 3B). On average, from 1 to 2.5 Hz, F_{dia} increase by 1.56 ± 0.42 mN/mm^2 under control conditions, but only 0.70 ± 0.19 at 10^{-7} M levosimendan ($P < 0.05$) and to 0.60 ± 0.22 mN/mm^2 at 10^{-5} M ($P < 0.05$ vs. control). In the group with the positive force–frequency relationship, the change in stimulation frequency to 2.5 Hz amounted in a change in F_{dia} from 0.94 ± 0.49 mN/mm^2 (control) to 0.41 ± 0.21 at 10^{-7} M levosimendan ($P < 0.05$) and to 0.39 ± 0.39 mN/mm^2 at 10^{-5} M ($P < 0.05$ vs. control). In the group with the negative force–frequency relationship, F_{dia} changed from 2.02 ± 0.64 mN/mm^2 (control) to 0.95 ± 0.30 at 10^{-7} M levosimendan ($P < 0.05$) and to 0.75 ± 0.27 mN/mm^2 at 10^{-5} M ($P < 0.05$ vs. control). Thus, levosimendan clearly attenuated diastolic dysfunction that was initiated by the increased stimulation frequency.

Upon increase in the stimulation frequency, time to peak tension was reduced (faster) in both groups ($P < 0.05$), as was time from peak tension to 50% and 90% relaxation ($P < 0.05$). Levosimendan did not alter this frequency-dependent shortening of the contraction. This implies that the speed of relaxation was not impaired nor improved at 2.5 Hz stimulation frequency compared to control, both in the group with the negative force–frequency relationship and in the preparations with an initial positive force–frequency relationship.

4. Discussion

The present study shows that the Ca^{2+}-sensitizer levosimendan improves function of failing human myocardium. Levosimendan, despite its Ca^{2+}-sensitizing properties, not only prevented a worsening of diastolic dysfunction; it even improved diastolic properties of the myocardium. In addition, systolic function improved in presence of therapeutically relevant concentrations of levosimendan.

4.1. Mechanism of action

Ca^{2+}-sensitizers act downstream of the sarcoplasmic reticulum Ca^{2+} handling process but can interact on different processes of the contractile apparatus. In contrast to EMD 53998, EMD 57033, and caffeine (Solaro et al., 1993; Haikala et al., 1995a; Wahr and Metzger, 1999) levosimendan, pimobendan, and bepridil do not impact on myosin ATPase activity, leaving cross-bridge cycling unaffected (Haikala and Linden, 1995; Hasenfuss et al., 1995;

Wahr and Metzger, 1999). The Ca^{2+}-sensitizing effect of levosimendan has been shown to occur through a direct interaction of the drug with troponin-C (Haikala et al., 1995b,c; Pollesello et al., 1994). However, a recent study failed to show binding to free troponin-C, and Ca^{2+} binding was not affected in nuclear magnetic resonance-experiments (Kleerekoper and Putkey, 1999), although prolonged incubation of the drug did seem to covalently bind to troponin-C. The Ca^{2+}-sensitizing effect of levosimendan has been hypothesized to be Ca^{2+}-dependent (Haikala and Linden, 1995), and would thus only exert its interaction with troponin-C in presence of sufficient amounts of Ca^{2+}. The mechanism responsible for the improvement of diastolic function is less understood, and it may very well be that the phosphodiesterase-III inhibitor activity is responsible. Others (Zimmermann et al., 1998; Sato et al., 1998) have postulated the phosphodiesterase-III inhibitory effect to play a role during diastole. Alternatively, it was shown that levosimendan increases activity of the Na^{+}–Ca^{2+} exchanger that may also contribute to improved relaxation (Hasenfuss et al., 1998).

4.2. Levosimendan and systolic function

Similar to previous work by us (Hasenfuss et al., 1998), and others (Haikala and Linden, 1995; Sato et al., 1998), levosimendan dose-dependently enhanced contractility. Interestingly, in preparations that had a positive force–frequency relationship, the enhancement of F_{dev} by levosimendan was more pronounced compared to preparations with a negative force–frequency relationship. This may indicate that the effect of levosimendan on Ca^{2+}-sensitivity is more pronounced in preparations with higher Ca^{2+} transients.

4.3. Levosimendan and diastolic function

The major clinical limitation of increased Ca^{2+}-sensitivity is that the myofilaments may be activated at low prevailing intracellular Ca^{2+} levels. In human heart failure with high diastolic Ca^{2+} levels this may result in a baseline active contraction, preventing complete relaxation of the heart and thus worsening diastolic function (Hajjar and Gwathmey, 1991). Besides systolic function impairment, diastolic dysfunction occurs in end-stage heart failure (Grossman, 1990; Arai et al., 1993; Gwathmey et al., 1990; Mulieri et al., 1982). This implies that these patients already have elevated resting Ca^{2+} levels, and diastolic myofilament activity may be even more pronounced. This has recently been shown in multicellular preparations by Hajjar et al. (1997); both EMD 57033 (without phosphodiesterase inhibition properties) and Org 30029 (with phosphodiesterase inhibition properties) impaired relaxation to a greater extend in failing human hearts than in non-failing human hearts.

It is now demonstrated that the negative effects of Ca^{2+}-sensitizers on diastolic function are not observed with levosimendan. Moreover, levosimendan improved diastolic function when diastolic Ca^{2+} levels where elevated with a physiological relevant protocol, i.e. via an increase in stimulation frequency. This indicated that the negative force–frequency behavior, a phenotype that has been shown to be present in end-stage heart failure (Pieske et al., 1995), could significantly be improved. Interestingly, in preparations that displayed a negative relationship, diastolic dysfunction improved more pronounced than in preparations with a positive relationship.

Relaxation time was significantly improved (i.e. abbreviated) by levosimendan in preparations with a negative force–frequency relationship. The accelerated relaxation partially "normalized" the existing differences between the groups. In preparations where the increase in F_{dev} was substantially larger than average, twitch timing parameters were however not affected. This could be due to the fact that increased force development in cardiac muscle preparations per se increases relaxation times (Janssen and Hunter, 1995). Thus, large inotropic responses may counterbalance the improvement in relaxation observed with levosimendan. In fact, different effects on twitch timing are in agreement with previous studies. In mammalian papillary muscles and myocytes, levosimendan did not prolong relaxation time (Haikala and Linden, 1995; Sato et al., 1998), but in end-stage failing human preparations (negative force–frequency relationship) levosimendan improved relaxation (Hasenfuss et al., 1998).

4.4. Clinical implications

The results of this study may be of clinical relevance because effects were observed under concentrations that may be clinically relevant (10^{-7} M). Levosimendan does not worsen diastolic function and can even partly reverse existing diastolic dysfunction (this study). From an energetic point of view, levosimendan is more economical compared to catecholamines or other inotropic interventions (Hasenfuss et al., 1995; Hasenfuss et al., 1996). Levosimendan does not "waste" energy for increased Ca^{2+} cycling and does not change cross-bridge kinetics uneconomically (Hasenfuss et al., 1995; Haikala et al., 1995a). Our results support the outcome of clinical studies; levosimendan has been shown to enhance cardiac performance (Lilleberg et al., 1998) and this without increased occurrence of arrhythmias (Nijhawan et al., 1999).

4.5. Limitations of the study

Although the results show a significant improvement in both systolic and diastolic function, we cannot discriminate whether the observed improvements are mediated through the Ca^{2+}-sensitizing effect of levosimendan alone, or

whether the phosphodiesterase-III inhibition effect also plays a role (Zimmermann et al., 1998; Sato et al., 1998). However, it was suggested that phosphodiesterase-III inhibition might be of minor relevance to the effects of levosimendan on myocardial function (Hasenfuss et al., 1995).

In this study, we have only investigated preparations from end-stage failing hearts. However, (i) we show that preparations with a positive force–frequency relationship, the positive effect on systolic and diastolic function are present, and at least equally as strong, and (ii) studies on healthy animal tissue reported similar positive inotropic effects (Haikala and Linden, 1995; Sato et al., 1998). Thus, we have reason to believe that the effects in patients suffering from lesser degrees of cardiac dysfunction may be similar to the results obtained in this study.

4.6. Conclusion

The Ca^{2+}-sensitizer levosimendan does not impair cardiac relaxation, and even improves diastolic function under situations where increased intracellular Ca^{2+} concentrations prevail. Levosimendan improved the force–frequency relationship, increased systolic force development, and decreased relaxation time in preparations with a negative force–frequency relationship.

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