Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 507 (2005) 199–209

www.elsevier.com/locate/ejphar

Contractile action of levosimendan and epinephrine during acidosis

Wolfgang Toller[a], Gerald Wölkart[b], Christian Stranz[a], Helfried Metzler[a], Friedrich Brunner[b,*]

[a]Department of Anaesthesiology and Intensive Care Medicine, Medical University of Graz, Auenbruggerplatz 29, 8036-Graz, Austria
[b]Department of Pharmacology and Toxicology, University of Graz, Universitätsplatz 2, 8010-Graz, Austria

Received 30 September 2004; received in revised form 18 November 2004; accepted 23 November 2004
Available online 24 December 2004

Abstract

We evaluated the inotropic actions of levosimendan and epinephrine, both singly and in combination, under isohydric (pH 7.4) and acidotic (pH 7.0) conditions in isolated guinea-pig hearts. Acidosis depressed contractility and myocardial relaxation by 25–30%, and both inotropes were less efficacious at pH 7.0, while their potencies were unaffected. In combination experiments, the presence of levosimendan increased the potency of epinephrine ~17-fold (pH 7.4) and 11-fold (pH 7.0), and the presence of epinephrine increased the potency of levosimendan ~12-fold (pH 7.4) and ~21-fold (pH 7.0). At pH 7.0, both inotropes augmented papillary muscle contraction to a similar extent, but in contrast to epinephrine, levosimendan *significantly* raised cAMP levels. In conclusion, combining levosimendan with epinephrine helps to overcome the depressed inotropic actions of epinephrine during acidosis, suggesting that additional studies which might justify clinical evaluation of the concurrent use of the two agents should be performed.

Keywords: Levosimendan; Acidosis; Epinephrine; Guinea pig; Contractility; cAMP

1. Introduction

Many reports have shown that in states of acidosis myocardial contractility is impaired and the efficacy of positive inotropic drugs to attenuate or reverse acidosis-induced contractile impairment is substantially limited (Cingolani et al., 1975; Ford et al., 1968; Hindman, 1990; Wildenthal et al., 1968). The reasons for the decreased response to positive inotropic drugs and the underlying mechanisms of contractile impairment may be related to the mode and site of action of these drugs. In fact, most inotropic drugs increase myocardial contractility by elevating intracellular concentrations of cAMP and cytosolic Ca^{2+}, either through stimulation of ß-adreno receptors (catecholamines), or via inhibition of cAMP breakdown by inhibitors of phosphodiesterase (PDE) (Tanaka et al., 1998). During acidosis, a number of cellular changes occur, including downregulation of the number (Marsh et al., 1988) and affinity (Modest and Butterworth, 1995) of ß-adreno receptors, decreased activity of adenylate cyclase with subsequent decreased formation of cAMP (Nakanishi et al., 1987; Tanaka et al., 1998) and inhibition of Ca^{2+} exchange (Poole Wilson and Langer, 1979). Furthermore, there is good evidence to show that the responsiveness of myofilaments to Ca^{2+} is impaired in acidosis (Than et al., 1994; Williamson et al., 1976), which alone or together with the other changes may account for impaired pump function. Interestingly, several reports have shown that reduced myofilament sensitivity to Ca^{2+} may not only involve troponin C, but could also be due to increased phosphorylation of troponin I following ß-adreno stimulation or PDE inhibition (Solaro et al., 1976; Tavernier et al., 2001).

Recently, levosimendan, a new positive inotrope that improves the sensitivity of myofilaments to Ca^{2+} (Ca^{2+} sensitizer) by selectively binding to troponin C (Pollesello et al., 1994), has become available in several European countries for treatment of low-output heart failure (Follath

* Corresponding author. Tel.: +43 316 380 5559; fax: +43 316 380 9890.
E-mail address: friedrich.brunner@kfunigraz.ac.at (F. Brunner).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.11.049

et al., 2002) and left ventricular failure complicating myocardial infarction (Moiseyev et al., 2002). Levosimendan improves myocardial contractility without increasing intracellular Ca^{2+} or stimulating ß-adreno receptors, or substantially inhibiting phosphodiesterase. This novel mechanism may explain why levosimendan exerts favourable clinical effects in the absence of increased myocardial oxygen consumption (Lilleberg et al., 1998) or arrhythmias (Follath et al., 2002), in contrast to catecholamines. Whether acidosis weakens the positive inotropic actions of Ca^{2+} sensitizers is presently not known, nor whether such drugs might help in restoring contractility and improve the efficacy of catecholamines. Therefore, combining drugs acting through different mechanisms and sites of action might be a promising therapeutic approach to restore contractility in acidotic states.

In the current investigation we evaluated the positive inotropic effect of levosimendan under normal and acidotic conditions. We hypothesized that combining levosimendan with epinephrine improves contractile function under isohydric and/or acidotic conditions. Experiments were carried out in isolated Langendorff-perfused guinea-pig hearts and papillary muscles (cAMP measurements).

2. Materials and methods

2.1. Heart perfusion

Guinea pigs of either sex weighing 350–450 g were sacrificed by cervical dislocation. The hearts were rapidly excised, arrested in ice-cold Krebs–Henseleit perfusion medium (perfusate) and mounted within 2 min of thoracotomy. Retrograde perfusion (Langendorff mode) was established at a rate of 10 ml/min/g heart wet weight with a modified Krebs–Henseleit bicarbonate buffer (composition in mM: NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.0, $MgCl_2$ 1.2, D-Glucose 10.1; pH 7.4) using the ISOHEART perfusion system (Harvard Apparatus/Hugo Sachs Elektronik, March-Hugstetten, Germany). Perfusion solutions with acidotic pH were prepared by adding $NaHCO_3$ at lower concentration (11 mM); to compensate for this, NaCl was used at higher concentration (132.4 mM) than at pH 7.4. The pH value of the perfusates (7.0 and 7.4) was measured over 2 min at the aortic cannula with no heart attached, while the perfusate was pumped through the aortic cannula at the normal flow rate, oxygenation and temperature. The actual perfusion rate varied between 14 and 18 ml/min depending on the heart weights (1.4–1.75 g) which were obtained graphically from the relationship between body weight and heart weight (r=1.00 for the range of body weights used here). Perfusion medium was continuously exposed to 95% O_2 and 5% CO_2 in an oxygenation chamber. Heart temperature was measured (Physitemp Instruments, Clifton, NJ) and maintained at 37 °C during perfusion.

Cardiac parameters were monitored continuously and included left ventricular developed pressure, maximum rate of left ventricular pressure development and maximum rate of left ventricular pressure decline (all three via a left ventricular fluid-filled latex balloon), coronary perfusion pressure, and heart rate. Further details have been reported previously (Brunner, 1997). Protocols were approved by the Institutional Animal Care and Use Committee, and all procedures used were in accordance with NIH guidelines for animal care and use (NIH Publication No. 85-23, revised 1996). A total of 52 animals was used (heart perfusions and papillary muscles) and no hearts were excluded.

2.2. Experimental procedure

All hearts were equilibrated at pH 7.4 for 45 min at constant flow and baseline functional parameters recorded. Experiments were then carried out either at isohydric (pH 7.4) or acidotic (pH 7.0) conditions using a total of 8 sets of hearts in eight different protocols. A first set of hearts (pH 7.4, n=6) was perfused with rising concentrations of epinephrine (1 nM–3 μM final concentration), *each added as bolus over 5–10 s*, via sideline in non-cumulative manner. For each concentration, functional parameters were documented at their maximum, which was attained at ~2 min after addition of the bolus dose. Once the effect tended to decline again, the next dose was added. A second set of hearts (pH 7.4, n=6) was similarly perfused and the effects of levosimendan (10 nM–10 μM added non-cumulatively) were documented. These experiments were repeated at pH 7.0 (sets 3 and 4, n=6). All other experiments were combination experiments carried out either at pH 7.4 or pH 7.0. When testing the effect of levosimendan, a fixed concentration of levosimendan (3 μM) was administered for 5 min (marked as B* in Figs. 1–3, panels C and E and Fig. 4A and C), followed by addition of rising bolus doses of epinephrine (1 nM–3 μM) in the continued presence of levosimendan (set 5: pH 7.4, n=4; set 6: pH 7.0, n=7). Finally, a fixed concentration of epinephrine (100 nM) was administered for 5 min (indicated as B* in Figs. 1–3, panels D and F and Fig. 4B and D), followed by addition of rising bolus doses of levosimendan (10 nM–10 μM) in the continued presence of epinephrine (set 7: pH 7.4, n=4; set 8: pH 7.0, n=8). A relatively low concentration of epinephrine (100 nM) and a relatively high concentration of levosimendan (3 μM) were chosen for co-administration with the respective other drug, because epinephrine is considerably more efficacious (E_{max}) than levosimendan, at similar affinity (compare Table 1). This way, the combination effect could be analyzed in terms of both parameters, EC_{50} and E_{max}.

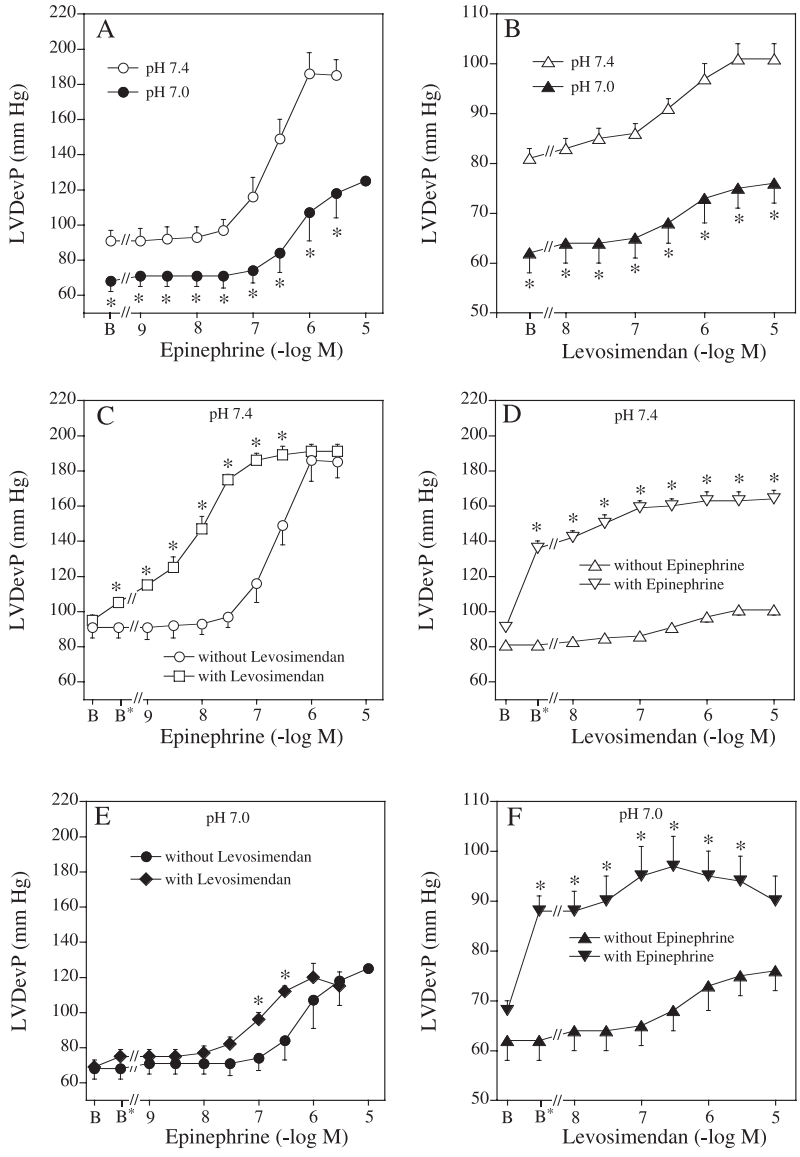

Fig. 1. Left ventricular developed pressure (LVDevP) data for epinephrine (A, C, E) and levosimendan (B, D, F) at pH 7.4 (open symbols) or pH 7.0 (closed symbols). Panels C–F show agonist combination experiments, in which baseline (see B on abscissa) was established at the respective pH value, followed by infusion over 5 min of the co-administered drug 3 μM levosimendan (C, E) or 100 nM epinephrine (D, F). The effect of the co-administered drug is indicated as B* on the abscissa. Data are mean±S.E.M. (for number of experiments, see Tables 1–3). * $P<0.05$ vs. respective values at pH 7.4 (A, B) or $P<0.05$ single drug vs. both drugs combined (C–F). Note different ordinate scale in panels B and F.

The rationale for the dual protocol (infusion of one drug at fixed dose throughout the experiment, and rising bolus doses of the other drug) was to assure correct quantification of the combination effect. To avoid incomplete functional effects due to differences in drug kinetics (epinephrine is degraded much faster than levosimendan), one drug was infused until a steady effect level was achieved onto which the effect of the other drug was added. This procedure implies that the infused drug will accumulate and so produce a greater effect than when used as bolus. With respect to epinephrine, infusing the drug over 5 min delivers ~7.5 nmol (not counting degradation), whereas the corresponding bolus dose delivers only ~1.4 nmol. For this reason, the value for B* in panels D and F needs to be, and indeed is, significantly higher than the value for 100 nM epinephrine tested as bolus concentration (e. g., panel A, Fig. 1).

2.3. Papillary muscle experiments and cAMP analysis

Guinea-pig papillary muscles were excised and superfused in organ baths (5 ml) with Krebs–Henseleit medium as used in the other experiments. Tissues were equilibrated for 60 min at pH 7.4, the medium switched to pH 7.0, the new baseline established, and individual concentrations of

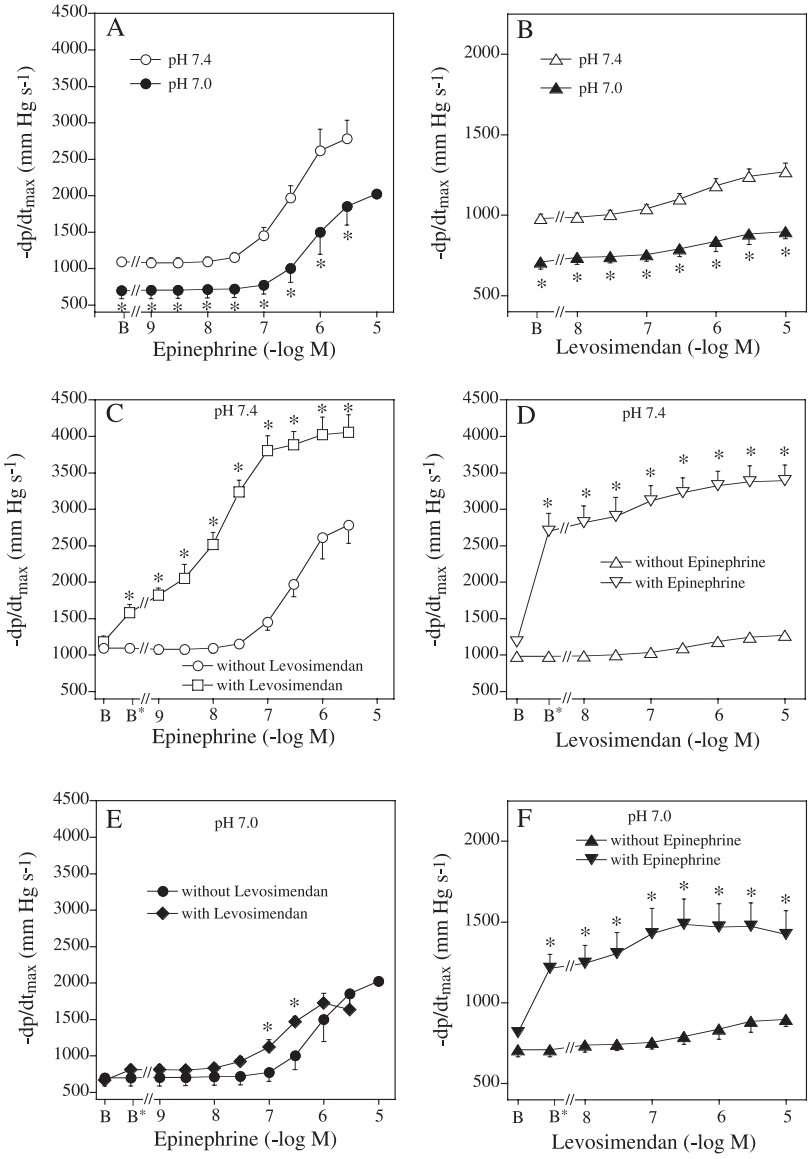

Fig. 2. Maximum rate of left ventricular pressure decline ($-dP/dt_{max}$) data for epinephrine (A, C, E) and levosimendan (B, D, F) at pH 7.4 (open symbols) or pH 7.0 (closed symbols). Panels C–F show agonist combination experiments, in which baseline (see B on abscissa) was established at the respective pH value, followed by infusion over 5 min of the co-administered drug 3 μM levosimendan (C, E) or 100 nM epinephrine (D, F). The effect of the co-administered drug is indicated as B* on the abscissa. Data are mean±S.E.M. (for number of experiments, see Tables 1–3). *P<0.05 vs. respective values at pH 7.4 (A, B) or P<0.05 single drug vs. both drugs combined (C–F). Note different ordinate scale in panels B and F.

agonist or both agonists together were applied and developed force registered. Diastolic force was set at 0.65 g. Tissues were freeze-clamped, cAMP extracted and subjected to radioimmunoassay using standard methodology.

2.4. Methods

Levosimendan (*R*-[[4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)pheny]hydrazono]propanedinitrile) was a generous gift of Orion Famos (Espoo, Finland), epinephrine hydrochloride was from Aventis (Frankfurt, Germany). All other chemicals were of the finest grade available. All concentrations given are final.

2.5. Data analysis

Group data are presented as arithmetic mean values±S.E.M. unless specified otherwise. Half-maximum effective concentration (EC_{50}) and maximum effect (E_{max}) values were obtained for each concentration–response curve with the use of a five-parameter logistic function using Kaleidagraph curve-fitting software on an Apple Macintosh Power PC. The logarithms of the EC_{50} values and the corresponding S.E.M. values were calculated and these values as well as the untransformed E_{max} values were compared by *t*-test. Differences were considered significant at P<0.05.

3. Results

3.1. Effects on ventricular contractility

Heart function was not different between experimental groups at baseline prior to switching to acidic solution. Under standard conditions (pH 7.4) epinephrine (1 nM–3 μM) increased left ventricular developed pressure from 91±6 mm Hg to a maximum of 186±12 mm Hg (2-fold; Fig. 1A) and maximum rate of left ventricular pressure development from 1017±51 to 3733±162 mm Hg/s (3.7-fold; not shown). Lowering pH to 7.0 reduced baseline contractility (left ventricular developed pressure: 68±6 mm Hg; 75%) as well as the contractile effects of epinephrine on this parameter (Fig. 1A) and the maximum rate of left ventricular pressure development (not shown). Statistical treatment of the data revealed that the agonist's efficacy (E_{max}), but not its potency (EC_{50}) was significantly reduced (Table 1). The effect of levosimendan (10 nM–10 μM) is shown in Fig. 1B. Levosimendan increased left ventricular developed pressure from 81±2 mm Hg to a maximum of 101±3 mm Hg (1.2-fold) and the rate of pressure development from 957±41 to 1423±78 mm Hg/s (1.5-fold; not shown). Acidosis significantly reduced baseline left ven-

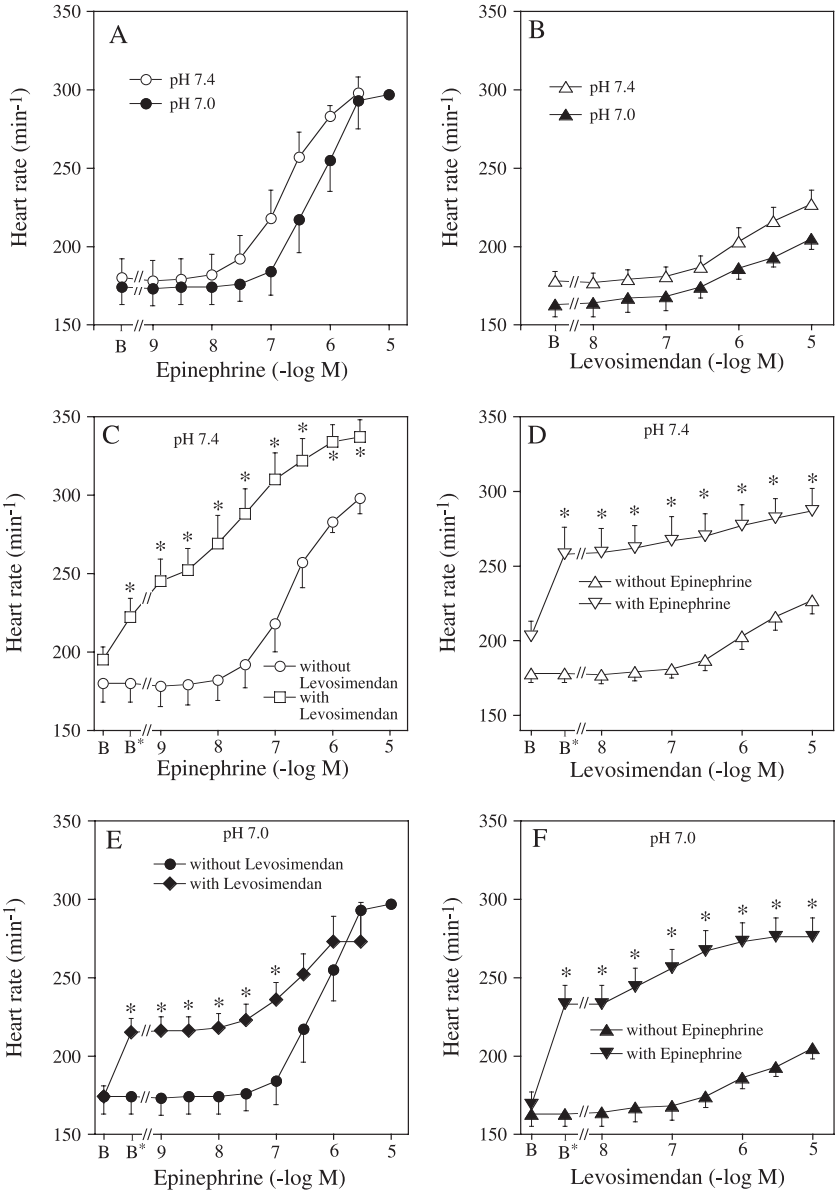

Fig. 3. Heart rate data for epinephrine (A, C, E) and levosimendan (B, D, F) at pH 7.4 (open symbols) or pH 7.0 (closed symbols). Panels C–F show agonist combination experiments, in which baseline (see B on abscissa) was established at the respective pH value, followed by infusion over 5 min of the co-administered drug 3 μM levosimendan (C, E) or 100 nM epinephrine (D, F). The effect of the co-administered drug is indicated as B* on the abscissa. Data are mean±S.E.M. (for number of experiments, see Tables 1–3). The greater effects of 3 μM levosimendan in panel E compared to panel B and of 100 nM epinephrine in panel F compared to panel A (in each case given as B*) results from infusing the agonist rather than adding it as a bolus (see also Methods). *P<0.05 vs. respective values at pH 7.4 (A, B) or P<0.05 single drug vs. both drugs combined (C–F).

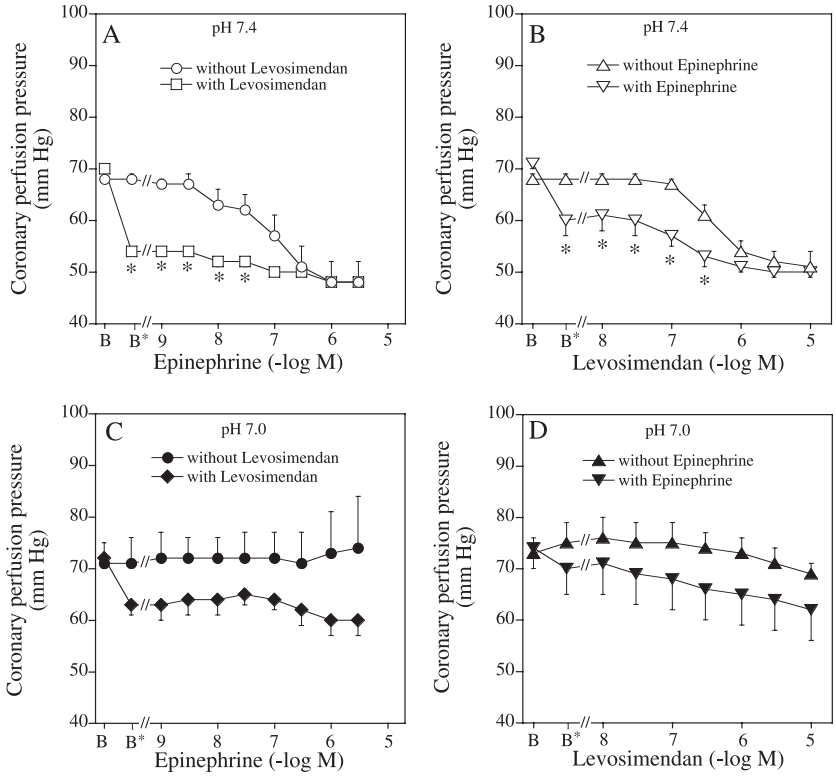

Fig. 4. Coronary perfusion pressure data in combination experiments at pH 7.4 (A, B) or pH 7.0 (C, D). Hearts were perfused with 3 μM levosimendan together with rising concentrations of epinephrine (A, C) or 100 nM epinephrine together with rising concentrations of levosimendan (B, D). The effect of the co-administered drug prior to start of the dose response curve of second agonist is again shown as B*. Data are mean±S.E.M. derived from 3–4 (A, B) or 7–8 (C, D) experiments, respectively. *P<0.05 single drug vs. both drugs combined. The significant concentration-dependent relaxing effect of epinephrine (A) and levosimendan (B) is not indicated separately. At pH 7.0 (C, D), group data were not significantly different. There were no significant differences between groups. B, baseline.

tricular developed pressure (62±4 mm Hg; 77%), and the positive inotropic effects of levosimendan. As in the case of epinephrine, the agonist's efficacy, but not its potency, was reduced (Table 1).

To evaluate the potential advantage of combining the two drugs, we performed experiments both at pH 7.4 and 7.0. In view of its lesser intrinsic efficacy compared to epinephrine, levosimendan was used at a relatively high concentration in these combination experiments (3 μM), whereas for epinephrine a relatively low concentration (100 nM) was chosen to avoid obscuring the dose–effect relationship of the weaker agonist levosimendan (compare Fig. 1A and B). The data for pH 7.4 are shown in Fig. 1C and D. Levosimendan increased the contractility-enhancing effect of epinephrine 17.3-fold (left ventricular developed pressure, Fig. 1C) and 15.1-fold (maximum rate of left ventricular pressure development, not shown), respectively. Similarly, levosimendan increased the efficacy of epinephrine with respect to the velocity of ventricular pressure decline and heart rate (8.9- and 9.2-fold, respectively; compare Tables 1 and 2). Epinephrine increased the potency of levosimendan 12.1-fold (left ventricular developed pressure) and 45.2-fold (maximum rate of left ventricular pressure development) (Fig. 1D), respectively. As expected, E_{max} was also significantly affected for all parameters (Table 2).

In view of these results at pH 7.4, we combined levosimendan with epinephrine at pH 7.0. The data are given in Fig. 1E. In this protocol, the potency of epinephrine was increased 11-fold (P<0.05 vs. epinephrine alone), whereas E_{max} was not different. Similar results were obtained for maximum rate of left ventricular pressure development (Table 3). Finally, we also tested the effect of a single concentration of epinephrine (100 nM) on the reduced inotropic action of levosimendan at pH 7.0 (Fig. 1F). In this setting, epinephrine substantially increased baseline left ventricular developed pressure, and levosimendan-increased left ventricular developed pressure (and maximum rate of left ventricular pressure development; not shown) somewhat further, but the higher concentrations were progressively less effective. The corresponding parameter values are shown in Table 3. As in the case of pH 7.4, 100 nM epinephrine raised left ventricular developed pressure more potently (indicated as B* in Fig. 1D and F) than would be expected from its effect shown in Fig. 1A due to the infusion of the agonist over 5 min in the combination protocol, compared to 5–10 s in the other experiments (bolus application; Fig. 1A). The same observation was

Table 1
E_{max} and EC_{50} values of epinephrine and levosimendan at pH 7.4 and 7.0

Parameter	E_{max}[a]		EC_{50}[b]	
	Epi	Levo	Epi	Levo
LVDevP				
pH 7.4	172±12	101±3	$1.9\pm0.3\times10^{-7}$	$4.6\pm1.0\times10^{-7}$
pH 7.0	129±10[c]	76±4[c]	$1.3\pm0.6\times10^{-6}$	$9.6\pm2.3\times10^{-7}$
$+dP/dt_{max}$				
pH 7.4	3762±218	1458±75	$2.2\pm0.3\times10^{-7}$	$1.4\pm0.9\times10^{-6}$
pH 7.0	2846±207[c]	1074±95[c]	$1.2\pm0.5\times10^{-6}$	$8.9\pm2.3\times10^{-7}$
$-dP/dt_{max}$				
pH 7.4	2706±148	1318±66	$3.0\pm0.6\times10^{-7}$	$6.3\pm1.4\times10^{-7}$
pH 7.0	2035±169[c]	946±47[c]	$1.2\pm0.5\times10^{-6}$	$1.7\pm0.9\times10^{-7}$
HR				
pH 7.4	330±17	235±12	$3.1\pm1.1\times10^{-7}$	$1.8\pm0.5\times10^{-6}$
pH 7.0	308±11	196±41	$9.5\pm4.1\times10^{-7}$	$7.0\pm3.8\times10^{-6}$

Data are mean±S.E.M. of six hearts.
Epi, epinephrine; Levo, levosimendan; LVDevP, left ventricular developed pressure (mm Hg); $+dP/dt_{max}$, maximum rate of left ventricular pressure rise (mm Hg/s); $-dP/dt_{max}$, maximum rate of left ventricular pressure decline (mm Hg/s); HR, heart rate (beats/min).
[a] E_{max}, maximum effect.
[b] EC_{50}, half-maximum effective concentration (M).
[c] $P<0.05$ vs. pH 7.4.

made for maximum rate of left ventricular pressure decline and heart rate (see below).

3.2. Effects on ventricular relaxation

Under standard conditions (pH 7.4) epinephrine increased maximum rate of left ventricular pressure decline from 1093±46 (baseline) to 2780±250 mm Hg/s. Acidosis reduced baseline maximum rate of left ventricular pressure decline to 697±115 mm Hg/s (=64%) and shifted the epinephrine curve to the right (Fig. 2A). Statistical analysis of both curves revealed a similar potency, but reduced maximum effect (Table 1). The corresponding effects of levosimendan are shown in Fig. 2B. Both at pH 7.4 and 7.0, this agonist hastened relaxation by 1.3-fold, but the lower E_{max} at pH 7.0 was largely an effect of the reduced baseline relaxation velocity (see also Table 2).

The effects of combining the two drugs at pH 7.4 are shown in Fig. 2C and D. Levosimendan enhanced the effects of epinephrine, as evident from a 16.7-fold decrease in EC_{50} and a considerable increase in E_{max}. Similarly, epinephrine augmented the myocardial relaxation effects of levosimendan (EC_{50}, 6.5-fold; E_{max}, 3.6-fold ($P<0.05$ vs. respective drug alone) (Table 2). The effects of combining the two agonists at pH 7.0 are shown in Fig. 2E and F. Levosimendan shifted the concentration response-curve of epinephrine to the left ($P<0.05$), but had no effect on E_{max}. In contrast, epinephrine displaced the concentration–response curve of levosimendan upward, largely due to its effect on baseline, without affecting EC_{50}. (Table 3).

3.3. Effects on heart rate

Both agonists dose-dependently increased heart rate, but epinephrine considerably more so than levosimendan (Fig. 3A and B). Acidosis had no effect on baseline or maximum increases in heart rate. In the combination experiments at pH 7.4 and 7.0, baseline heart rate was raised by the co-

Table 2
E_{max} and EC_{50} values at pH 7.4 resulting from combining epinephrine (1 nM–10 μM) with a fixed concentration of levosimendan (3 μM), or levosimendan (10 nM–10 μM) with a fixed concentration of epinephrine (100 nM)

	E_{max}[a]	EC_{50}[b]
LVDevP		
Epi+3 μM Levo	191±5	$1.1\pm0.3\times10^{-8}$[c]
Levo+100 nM Epi	163±5[d]	$3.8\pm1.0\times10^{-8}$[d]
$+dP/dt_{max}$		
Epi+3 μM Levo	6703±454[c]	1.4 ± 0.3^{-8}[c]
Levo+100 nM Epi	5540±167[d]	$3.1\pm1.2\times10^{-8}$[d]
$-dP/dt_{max}$		
Epi+3 μM Levo	4033±236[c]	$1.8\pm0.4\times10^{-8}$[c]
Levo+100 nM Epi	3397±222[d]	$9.7\pm1.6\times10^{-8}$[d]
HR		
Epi+3 μM Levo	341±10	$3.7\pm1.7\times10^{-8}$[c]
Levo+100 nM Epi	289±15[d]	$8.7\pm2.1\times10^{-8}$[d]

Data are mean±S.E.M. of four hearts.
For abbreviations, see Table 1.
[a] E_{max}, maximum effect.
[b] EC_{50}, half-maximum effective concentration (M).
[c] $P<0.05$ vs. epinephrine alone.
[d] $P<0.05$ vs. levosimendan alone.

Table 3
E_{max} and EC_{50} values at pH 7.0 resulting from combining epinephrine (1 nM–10 μM) with a fixed concentration of levosimendan (3 μM), or levosimendan (10 nM–10 μM) with a fixed concentration of epinephrine (100 nM)

	E_{max}[a]	EC_{50}[b]
LVDevP		
Epi+3 μM Levo	119±7	$1.2\pm0.4\times10^{-7}$[c]
Levo+100 nM Epi	96±5[c]	$4.6\pm1.1\times10^{-8}$[d]
$+dP/dt_{max}$		
Epi+3 μM Levo	2363±130	$1.1\pm0.4\times10^{-7}$[c]
Levo+100 nM Epi	1661±111[d]	$3.6\pm0.8\times10^{-8}$[d]
$-dP/dt_{max}$		
Epi+3 μM Levo	1712±144	$1.6\pm0.5\times10^{-7}$
Levo+100 nM Epi	1470±147[d]	$6.1\pm1.5\times10^{-8}$
HR		
Epi+3 μM Levo	297±25	$3.4\pm1.3\times10^{-7}$
Levo+100 nM Epi	294±10[d]	$9.0\pm2.9\times10^{-8}$

Data are mean±S.E.M. of 7 and 8 hearts, respectively.
For abbreviations, see Table 1.
[a] E_{max}, maximum effect.
[b] EC_{50}, half-maximum effective concentration (M).
[c] $P<0.05$ vs. epinephrine alone.
[d] $P<0.05$ vs. levosimendan alone.

administered agonist, i.e. levosimendan (Fig. 3C and E) or epinephrine (Fig. 3D and F), respectively (indicated as B*). As in the case of the other parameters mentioned above, this is due to infusion of the agonists over 5 min. The statistical analysis showed no difference in EC_{50} or the E_{max} between pH values when drugs were administered singly (Table 1). As co-administered drug, levosimendan significantly shifted the heart rate curve of epinephrine to the left and upward at pH 7.4 and 7.0 (Fig. 3C and E), but curve-fitting analysis showed unchanged E_{max} and EC_{50} values (P=non-significant vs. either drug alone). On the other hand, epinephrine significantly increased E_{max} at both pH values, without effect on EC_{50} (Fig. 3D and F) (compare also the tables). We also quantified arrhythmias in the different protocols by analyzing the frequency trace in the different protocols. We noted no arrhythmias at normal pH, but at pH 7.0 occasional ectopic beats occurred toward the end of the protocol (7±4 extrasystoles during the last 10 min of acidosis). The highest concentrations of epinephrine (1–3 μM) provoked between 15 and 20 extrasystoles in two out of six hearts; four hearts showed no arrhythmias. In no case continuous periods of tachycardia of more than 5 sec were observed. Levosimendan had no effect on cardiac rhythm, even at the highest concentrations. In combination experiments, neither epinephrine (100 nM) nor levosimendan (3 μM) disturbed cardiac rhythm compared to pre-protocol baseline.

3.4. Effects on coronary perfusion pressure

In view of the known effects of the two test drugs on coronary vessels, complete dose–effect curves were performed under isohydric and acidotic conditions (Fig. 4). At pH 7.4, both drugs significantly reduced perfusion pressure in dose-dependent fashion (open circles in Fig. 4A and open triangles in Fig. 4B; statistical significance not indicated). The co-administered drug in itself substantially reduced perfusion pressure (indicated as B*), leaving both epinephrine (Fig. 4A) and levosimendan (Fig. 4B) without much additional effect ($P<0.05$ combined drugs vs. individual). Acidotic perfusion, however, suppressed the coronary vasodilatatory effect of both agonists Fig. 4C and D).

3.5. cAMP measurements

Because levosimendan potentially stimulates cAMP formation through inhibition of phosphodiesterase, we determined whether this action is crucial for the contraction-enhancing effects in our combination protocol. In view of the clinical perspective of our study, the experiments were mostly limited to pH 7.0. Acidosis reduced developed force in superfused papillary muscles from 0.62±0.1 to 0.50±0.1 g. Addition of epinephrine (300 nM) to the tissue bath raised generated force 1.6-fold, levosimendan (3 μM) 1.3-fold and the combination of both agents raised it also 1.6-fold (Fig. 5A). [When analyzed as difference between maximum generated force minus respective intra-experimental baseline (Δ-values), the combination effect was

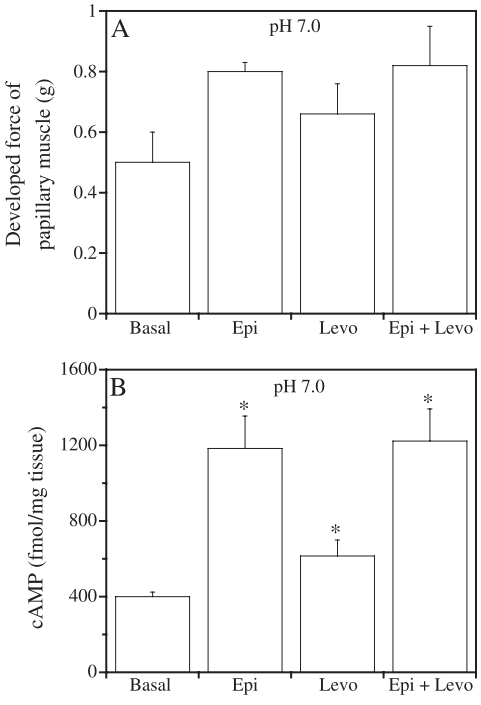

Fig. 5. Developed force data (A) and corresponding cyclic AMP tissue levels (B) of guinea pig papillary muscles at pH 7.0. Tissues were equilibrated for 30 min at pH 7.0. and generated force registered at baseline (Basal), following stimulation with 300 nM epinephrine (Epi), 3 μM levosimendan (Levo), or both drugs combined (Epi+Levo). For cAMP measurements, tissues were freeze-clamped 2 min after addition of agonist (Epi and Levo). Data are mean±S.E.M. derived from five experiments. *$P<0.05$ vs. basal.

significantly greater than the effect of epinephrine alone; data not shown.] The corresponding cAMP data are shown in Fig. 5B. Epinephrine as well as epinephrine together with levosimendan raised cAMP 3-fold, and levosimendan alone 1.5-fold ($P<0.05$ vs. baseline).

4. Discussion

Baseline contractility was reduced in acidosis by ~25% and epinephrine and levosimendan improved all contractile parameters both at pH 7.4 and 7.0. Combining levosimendan with epinephrine rendered the catecholamine more potent as an inotrope, and adding epinephrine increased the potency as well as the efficacy of levosimendan. Even the highest concentrations of epinephrine were unable to stimulate the heart up to levels observed in non-acidotic hearts, similar to previous observations with most other catecholamines (Andersen et al., 1967; Camilion de Hurtado et al., 1981; Huang et al., 1995; Kosugi and Tajimi, 1985; Marsh et al., 1988; Than et al., 1994). In one detailed analysis, norepinephrine and isoprenaline appeared to be as efficacious in acidosis (pH 6.85), whereas phenylephrine was less potent (Than et al., 1994).

Because a Ca^{2+} sensitizer (EMD-57033) was equally efficacious at pH 6.85 as at physiologic pH (Than et al., 1994), suggesting that acidosis may more effectively be overcome by reversing the Ca^{2+}-induced desensitization of myofilaments, we hypothesized that combining a Ca^{2+} sensitizing drug, such as levosimendan, with epinephrine would produce the desired inotropic effect at lower catecholamine doses. Recent studies done at pH 7.4 (Boknik et al., 1997; Brixius et al., 2002), but particularly the present results obtained at pH 7.0 (Figs. 1E and 2E) support this notion, because levosimendan increased the potency (EC_{50}) of epinephrine, albeit not its maximum effect. These novel findings may have clinical relevance, notably as an alternative strategy to current clinical practice, but this needs to be demonstrated in further studies. Similarly, the neutral effect of the combination therapy on heart rate (Fig. 3E) appears to be clinically attractive, despite the moderate heart rate raising effect of levosimendan observed in this and a previous study (Tassani et al., 2002). This view is also supported by studies in humans where levosimendan was shown not to increase myocardial oxygen consumption, which is particularly advantageous in states of decreased contractile reserve due to myocardial ischemia (Lilleberg et al., 1998). Hence, this regime may also prevent or lessen the risk of myocardial ischemia and arrhythmic events.

Levosimendan is increasingly being used clinically, but neither its efficacy in acidosis nor combination with other agents have been tested. The doses required to obtain maximum inotropy (1–10 μM) were higher than those of epinephrine, suggesting that levosimendan alone is a weaker agonist than epinephrine (compare Fig. 1B with A). In acidosis, the positive inotropic effect of levosimendan was attenuated, but the agonist's potency was unchanged (Table 1). As in this situation levosimendan raised developed pressure only up to ~75 mm Hg (Fig. 1B) and increased maximum rate of left ventricular pressure development only up to ~1000 mm Hg/s, but significantly shifted the concentration–response curve of epinephrine to the left, it may be a useful combination partner for epinephrine. Another aspect was that levosimendan, rather than reducing it, enhanced epinephrine-induced acceleration of maximum rate of left ventricular pressure decline which is in line with a recent experimental study (Sato et al., 1998).

The final aspect of our study relates to the usefulness of levosimendan as basic inotropic drug whose effectiveness is enhanced by addition of a catecholamine. This appears to be the clinically most important aspect. Our data show that combining the two agents in the way chosen here may in fact best lead to the desired graded response in patients with severely compromised myocardial function. These patients often do not tolerate exaggerated stimulation of myocardial contractility and heart rate inherent in catecholamine action, as evident from numerous studies in which beta-adreno antagonists improved survival in patients with myocardial infarction (Dargie, 2001; Mangano et al., 1996; Poldermans et al., 1999). Thus, it is conceivable that inotropic support in acidotic states should perhaps better rely on levosimendan as main inotrope, supplemented by the stronger catecholamine as far as necessary.

Another argument for combining these two drugs is provided by our cAMP measurements. In fact, the increase in potency of epinephrine with addition of levosimendan (Fig. 1E) appeared to be largely independent of cAMP, because papillary muscles generated identical force both on addition of epinephrine alone or in combination with levosimendan (Fig. 4). These data support the interpretation that the contractility-enhancing action of levosimendan in acidosis may primarily be due to Ca^{2+} sensitization which makes this drug suitable as a combination partner of catecholamines, unlike phosphodiesterase inhibitors such as milrinone. The view of two different modes of action (Fig. 4A) is not necessarily at odds with the similar cAMP levels elicited by epinephrine alone or in combination with levosimendan (Fig. 4B), because additive combinations may result "coincidentally" with agents exhibiting dose–response curves with greater steepness than those which compete for a single site (Pöch, 1993), which is observed in the present case (Fig. 1C and D).

In line with published reports we observed a significant coronary relaxant effect both for epinephrine (Nöhammer et al., 2003) and levosimendan (Gruhn et al., 1998; Krassoi et al., 2000) at normal pH. Presumably, epinephrine exerts vasodilation by activating vascular ß-receptors, whereas levosimendan has been shown to open vascular K_{ATP} channels (Yokoshiki et al., 1997). In our experiments, the vasoactive effects disappeared at pH 7.0, probably due to endothelial dysfunction (Giraldez et al., 1997).

Levosimendan produces an active metabolite, OR-1896, whose positive inotropic action is similarly reduced under acidotic conditions, e.g. in canine ventricular trabeculae, possibly due to a decreased Ca^{2+} availability (Takahashi and Endoh, 2002). We do not know whether the inotropic effects of levosimendan documented in this study were due to levosimendan itself or a metabolite like OR-1896.

In conclusion, we showed that myocardial acidosis of a clinically relevant level (pH 7.0) depresses basic contractile function in guinea-pig hearts and attenuates the positive inotropic actions of epinephrine and levosimendan. However, combining both drugs renders them significantly more potent and avoids their deficits as monotherapies. In spite of this, the true value of a combination strategy in a clinical setting can only be ascertained in patients.

Acknowledgements

The authors thank Petra Pierl and Eva Herk for technical assistance. Supported by the Austrian Science Fund (FWF), project 17339 (to F.B.).

References

Andersen, M.N., Border, J.R., Mouritzen, C.V., 1967. Acidosis, catecholamines and cardiovascular dynamics: when does acidosis require correction? Ann. Surg. 166, 344–356.

Boknik, P., Neumann, J., Kaspareit, G., Schmitz, W., Scholz, H., Vahlensieck, U., Zimmermann, N., 1997. Mechanisms of the contractile effects of levosimendan in the mammalian heart. J. Pharmacol. Exp. Ther. 280, 277–283.

Brixius, K., Reicke, S., Schwinger, R.H., 2002. Beneficial effects of the $Ca^{(2+)}$ sensitizer levosimendan in human myocardium. Am. J. Physiol, Heart Circ. Physiol. 282, H131–H137.

Brunner, F., 1997. Interaction of nitric oxide and endothelin-1 in ischemia/reperfusion injury of rat heart. J. Mol. Cell. Cardiol. 29, 2363–2374.

Camilion de Hurtado, M.C., Argel, M.I., Cingolani, H.E., 1981. Influence of acid–base alterations on myocardial sensitivity to catecholamines. Naunyn-Schmiedeberg's Arch. Pharmacol. 317, 219–224.

Cingolani, H.E., Faulkner, S.L., Mattiazzi, A.R., 1975. Depression of human myocardial contractility with respiratory and metabolic acidosis. Surgery 77, 427–432.

Dargie, H.J., 2001. Effect of carvedilol on outcome after myocardial infarction in patients with left-ventricular dysfunction: the CAPRICORN randomised trial. Lancet 357, 1385–1390.

Follath, F., Cleland, J.G., Just, H., Papp, J.G., Scholz, H., Peuhkurinen, K., Harjola, V.P., Mitrovic, V., Abdalla, M., Sandell, E.P., Lehtonen, L., 2002. Efficacy and safety of intravenous levosimendan compared with dobutamine in severe low-output heart failure (the LIDO study): a randomised double-blind trial. Lancet 360, 196–202.

Ford, G.D., Cline, W.H., Fleming Jr., W.W., 1968. Influence of lactic acidosis on cardiovascular response to sympathomimetic amines. Am. J. Physiol. 215, 1123–1129.

Giraldez, R.R., Panda, A., Xia, Y., Sanders, S.P., Zweier, J.L., 1997. Decreased nitric-oxide synthase activity causes impaired endothelium-dependent relaxation in the postischemic heart. J. Biol. Chem. 272, 21420–21426.

Gruhn, N., Nielsen-Kudsk, J.E., Theilgaard, S., Bang, L., Olesen, S.P., Aldershvile, J., 1998. Coronary vasorelaxant effect of levosimendan, a new inodilator with calcium-sensitizing properties. J. Cardiovasc. Pharmacol. 31, 741–749.

Hindman, B.J., 1990. Sodium bicarbonate in the treatment of subtypes of acute lactic acidosis: physiologic considerations. Anesthesiology 72, 1064–1076.

Huang, Y.G., Wong, K.C., Yip, W.H., McJames, S.W., Pace, N.L., 1995. Cardiovascular responses to graded doses of three catecholamines during lactic and hydrochloric acidosis in dogs. Br. J. Anaesth. 74, 583–590.

Kosugi, I., Tajimi, K., 1985. Effects of dopamine and dobutamine on hemodynamics and plasma catecholamine levels during severe lactic acid acidosis. Circ. Shock 17, 95–102.

Krassoi, I., Pataricza, J., Kun, A., Papp, J.G., 2000. Calcium-dependent vasorelaxant capacity of levosimendan in porcine and human epicardial coronary artery preparations. Cardiovasc. Drugs Ther. 14, 691–693.

Lilleberg, J., Nieminen, M.S., Akkila, J., Heikkila, L., Kuitunen, A., Lehtonen, L., Verkkala, K., Mattila, S., Salmenpera, M., 1998. Effects of a new calcium sensitizer, levosimendan, on haemodynamics, coronary blood flow and myocardial substrate utilization early after coronary artery bypass grafting. Eur. Heart J. 19, 660–668.

Mangano, D.T., Layug, E.L., Wallace, A., Tateo, I., 1996. Effect of atenolol on mortality and cardiovascular morbidity after noncardiac surgery. Multicenter study of perioperative ischemia research group. N. Engl. J. Med. 335, 1713–1720.

Marsh, J.D., Margolis, T.I., Kim, D., 1988. Mechanism of diminished contractile response to catecholamines during acidosis. Am. J. Physiol. 254, H20–H27.

Modest, V.E., Butterworth, J.F.t., 1995. Effect of pH and lidocaine on beta-adreno receptor binding. Interaction during resuscitation? Chest 108, 1373–1379.

Moiseyev, V.S., Poder, P., Andrejevs, N., Ruda, M.Y., Golikov, A.P., Lazebnik, L.B., Kobalava, Z.D., Lehtonen, L.A., Laine, T., Nieminen, M.S., Lie, K.I., 2002. Safety and efficacy of a novel calcium sensitizer, levosimendan, in patients with left ventricular failure due to an acute myocardial infarction. Eur. Heart J. 23, 1422–1432.

Nakanishi, T., Okuda, H., Kamata, K., Seguchi, M., Nakazawa, M., Takao, A., 1987. Influence of acidosis on inotropic effect of catecholamines in newborn rabbit hearts. Am. J. Physiol. 253, H1441–H1448.

Nöhammer, C., Brunner, F., Wölkart, G., Staber, P.B., Steyrer, E., Gonzalez, F.J., Zechner, R., Hoefler, G., 2003. Myocardial dysfunction and male mortality in peroxisome proliferator-activated receptor alpha knockout mice overexpressing lipoprotein lipase in muscle. Lab. Invest. 83, 259–269.

Pöch, G., 1993. Combined Effects of Drugs and Toxid Agents. Modern Evaluation in Theory and Practice. Springer, Wien, NY.

Poldermans, D., Boersma, E., Bax, J.J., Thomson, I.R., van de Ven, L.L., Blankensteijn, J.D., Baars, H.F., Yo, T.I., Trocino, G., Vigna, C., Roelandt, J.R., van Urk, H., 1999. The effect of bisoprolol on perioperative mortality and myocardial infarction in high-risk patients undergoing vascular surgery. Dutch echocardiographic cardiac risk evaluation applying stress echocardiography study group. N. Engl. J. Med. 341, 1789–1794.

Pollesello, P., Ovaska, M., Kaivola, J., Tilgmann, C., Lundstrom, K., Kalkkinen, N., Ulmanen, I., Nissinen, E., Taskinen, J., 1994. Binding of a new Ca^{2+} sensitizer, levosimendan, to recombinant human cardiac troponin C. A molecular modelling, fluorescence probe, and proton nuclear magnetic resonance study. J. Biol. Chem. 269, 28584–28590.

Poole Wilson, P.A., Langer, G.A., 1979. Effects of acidosis on mechanical function and Ca^{2+} exchange in rabbit myocardium. Am. J. Physiol. 236, H525–H533.

Sato, S., Talukder, M.A., Sugawara, H., Sawada, H., Endoh, M., 1998. Effects of levosimendan on myocardial contractility and Ca^{2+} transients in aequorin-loaded right-ventricular papillary muscles and indo-1-loaded single ventricular cardiomyocytes of the rabbit. J. Mol. Cell. Cardiol. 30, 1115–1128.

Solaro, R.J., Moir, A.G.J., Perry, S.V., 1976. Phosphorylation of troponin I and the inotropic effect of adrenaline in the perfused rabbit heart. Nature 262, 615–616.

Takahashi, R., Endoh, M., 2002. Effects of OR-1896, a metabolite of levosimendan, on force of contraction and Ca^{2+} transients under acidotic condition in aequorin-loaded canine ventricular myocardium. Naunyn-Schmiedeberg's Arch. Pharmacol. 366, 440–448.

Tanaka, M., Ishikawa, T., Nishikawa, T., Goto, K., Sato, S., 1998. Influence of acidosis on cardiotonic effects of milrinone. Anesthesiology 88, 725–734.

Tassani, P., Schad, H., Heimisch, W., Bernhard-Abt, A., Ettner, U., Mendler, N., Lange, R., 2002. Effect of the calcium sensitizer levosimendan on the performance of ischaemic myocardium in anaesthetised pigs. Cardiovasc. Drugs Ther. 16, 435–441.

Tavernier, B., Li, J.M., El Omar, M.M., Lanone, S., Yang, Z.K., Trayer, I.P., Mebazaa, A., Shah, A.M., 2001. Cardiac contractile impairment associated with increased phosphorylation of troponin I in endotoxemic rats. FASEB J. 15, 294–296.

Than, N., Shah, N., White, J., Lee, J.A., Orchard, C.H., 1994. Effects of acidosis and hypoxia on the response of isolated ferret cardiac muscle to inotropic agents. Cardiovasc. Res. 28, 1209–1217.

Wildenthal, K., Mierzwiak, D.S., Myers, R.W., Mitchell, J.H., 1968. Effects of acute lactic acidosis on left ventricular performance. Am. J. Physiol. 214, 1352–1359.

Williamson, J.R., Safer, B., Rich, T., Schaffer, S., Kobayashi, K., 1976. Effects of acidosis on myocardial contractility and metabolism. Acta Med. Scand., Suppl. 587, 95–112.

Yokoshiki, H., Katsube, Y., Sunagawa, M., Sperelakis, N., 1997. Levosimendan, a novel Ca^{2+} sensitizer, activates the glibenclamide-sensitive K+ channel in rat arterial myocytes. Eur. J. Pharmacol. 333, 249–259.