Br. J. Pharmacol. (1994), **112**, 757–762

A quantitative comparison of functional and anti-ischaemic effects of the phosphodiesterase-inhibitors, amrinone, milrinone and levosimendan in rabbit isolated hearts

[1]A.F.E. Rump, D. Acar & W. Klaus

Institut für Pharmakologie, Universität Köln, Gleuelerstr. 24, 50931 Köln, Germany

1 The functional and anti-ischaemic effects of the phosphodiesterase (PDE)-inhibitors, amrinone, milrinone and levosimendan, a new agent combining PDE-inhibitory with calcium-sensitizing properties, were investigated in rabbit isolated hearts (Langendorff, constant pressure: 70 cmH_2O, Tyrode solution, Ca^{2+} 1.8 mmol l^{-1}, 37°C). Anti-ischaemic effects were studied in electrically-driven hearts (200 beats min^{-1}). Acute regional ischaemia was induced by ligature of a branch of the circumflex coronary artery and quantified from epicardial NADH-fluorescence photography.

2 Cumulative concentration-response curves in spontaneously beating hearts in the presence of isoprenaline (10^{-10} M), showed a higher inotropic and coronary vasodilator potency for levosimendan (EC_{50}: 7×10^{-7} M) compared to milrinone (EC_{50}: 7.7×10^{-6} M) or amrinone (EC_{50}: 2×10^{-5} M). Although the maximal coronary dilator activity was similar for the three agents, the maximal inotropic and chronotropic effects were lower for levosimendan than for amrinone or milrinone ($P<0.05$).

3 In regionally ischaemic hearts, milrinone (10^{-5} M) or levosimendan (5×10^{-6} M) similarly enhanced the left ventricular pressure (+15–20%) ($P<0.05$) and the global coronary flow (+40–50%) ($P<0.05$). The epicardial NADH-fluorescence area was significantly diminished by milrinone or levosimendan (−20–30%) ($P<0.05$) and there was no significant difference between the anti-ischaemic effects of either agent ($P>0.05$).

4 It is concluded that amrinone and milrinone possess similar functional profiles in rabbit isolated hearts and a higher inotropic and chronotropic efficacy than levosimendan. At functionally equieffective concentrations, milrinone and levosimendan show similar anti-ischaemic effects, related to an improvement of myocardial perfusion. The calcium-sensitizing properties seem not to be relevant for cardioprotection by levosimendan at the concentration used.

Keywords: NADH-fluorescence; myocardial ischaemia; cardioprotection; inotropes; phosphodiesterase-inhibitors; calcium-sensitizers; amrinone; milrinone; levosimendan

Introduction

Phosphodiesterase (PDE)-inhibitors represent a class of agents combining inotropic and vasodilator properties (Leyen *et al.*, 1989). Several of these agents have been shown to possess anti-ischaemic properties that could be demonstrated in experimental (Jentzer *et al.*, 1981; Rump *et al.*, 1993a,e) and clinical situations (Benotti *et al.*, 1980). These anti-ischaemic effects have often been explained by peripheral dilatation and a reduction in myocardial oxygen-consumption. Moreover, it has been suggested that an improvement of myocardial perfusion might also contribute to the beneficial effects observed in isolated heart preparations (Rump *et al.*, 1993a,e). Thus, the relation of inotropic effects to vasodilator effects seems to be of prime importance for the anti-ischaemic properties of PDE-inhibitors. It was, however, demonstrated that PDE-inhibitors may differ in their influence on intracellular calcium recirculation (Morner, 1990; Holmberg *et al.*, 1991). Moreover, several agents combining PDE-inhibitory and calcium-sensitizing properties have been described (Kitzen & Winbury, 1989; Herzig & Quast, 1992). The sensitization of the myofilaments to calcium may not only represent a new inotropic principle; theoretically, a calcium-sensitizing effect might be accompanied by an oxygen-sparing effect (Van Zwieten, 1991). Therefore, PDE-inhibitors with calcium-sensitizing properties might possibly be expected to be particularly effective as anti-ischaemic inotropes.

Simendan is a novel compound combining PDE-inhibitory and calcium-sensitizing properties (Edes *et al.*, 1992; Haikala *et al.*, 1992a,b; Ovaska *et al.*, 1992; Raasmuja *et al.*, 1992). It was demonstrated that simendan augmented the tension developed by guinea-pig papillary muscles and chemically skinned fibres to the same extent in micromolar concentrations (100% tension increase at 3×10^{-6} M) (Haikala *et al.*, 1992a). In comparison, milrinone had no effect on the skinned fibres up to 10^{-4} M (Haikala *et al.*, 1992a). These findings indicate that at a micromolar concentration the positive inotropic action of simendan is actually caused by an increased calcium-sensitivity of contractile proteins and not by an increased calcium influx (Haikala *et al.*, 1992a). The precise mechanisms involved at the molecular level are still unclear, but it was shown that simendan binds to troponin (Ovaska *et al.*, 1992) and does not enhance myosin ATPase activity (Haikala *et al.*, 1992b). Although a potential drawback in the use of myofilament sensitizers is the possibility that they may impair diastolic function (Katz, 1986; Ventura *et al.*, 1992), it was shown that simendan does not delay relaxation (Haikala *et al.*, 1992b). Moreover, an inhibitory action on the voltage-sensitive Ca^{2+} current may explain the antiarrhythmic effects observed (Raasmaja *et al.*, 1992). Thus, several mechanisms of action may confer cardioprotective properties to simendan.

In the present study, we have investigated the functional and anti-ischaemic effects of levosimendan, the active enantiomer of simendan, in comparison to the 'pure' bipyridine-type PDE-inhibitors, amrinone and milrinone, in a rabbit isolated heart preparation.

Methods

Hearts from male rabbits (White New Zealand) (1.6–2.0 kg body weight) were prepared as published previously (Rump

[1] Author for correspondence.

et al., 1993d) and perfused according to Langendorff (Langendorff, 1895) at a constant pressure of 70 cmH_2O with Tyrode solution equilibrated with 95% O_2 and 5% CO_2 at 37°C. The Tyrode solution had the following millimolar composition: Na^+ 161, K^+ 5.36, Ca^{2+} 1.8, Mg^{2+} 1.05, Cl^- 148, HCO_3^- 23.8, $H_2PO_4^-$ 0.42, glucose 10. Left ventricular pressure was measured continuously with a pressure transducer (Trantec Modell 800, Bentley) attached to a fluid-filled rubber balloon which was inserted via the left atrium into the left ventricle. The volume was adapted to give an end-diastolic pressure of 0 mmHg. Left ventricular pressure was measured as actively developed left ventricular pressure, i.e. systolic minus end-diastolic left ventricular pressure. Global coronary flow was measured with graded glass cylinders. The hearts were electrically-driven (Stimulator-S, Hugo-Sachs Elektronik) as described in the experimental protocol.

For quantification of the ischaemic myoepicardium, photographs using endogenous NADH-fluorescence were taken and submitted to image processing (Rump et al., 1993d). The excitation light was provided by a Xe-flash (100 J in 120 μs, model Strobe 1001 S.O. 1, Drello), the light filtered through an excitation filter (UG1, 0.5 mm, Schott) and directed towards the heart by a 4 point quartz light guide (diameter 10 mm, Volpi). A selected pulse of the electrical stimulator was used to trigger the flash lights without delay so that the pictures were always taken during the same phase of the cardiac contraction cycle (late ventricular diastole). NADH-fluorescence was recorded in a dark room on high sensitivity film (Polaroid type 667, 36 DIN) with a cut on emission filter (GG 435/3 mm, Schott) in front of a Rolleiflex SL66 camera (shutter open) fitted with an 80 mm retro lens and bellow attachment which allowed an image magnification of up to 1.5.

Epicardial NADH-fluorescence photographs were digitized into a matrix of 768 × 512 pixels and 255 grey levels, and image processing was performed on a Lion 486 PC (CCD-video-camera 1000 WOL, Mintron Enterprise Ltd; frame graver board, Data translation, Marlboro, U.S.A.; JAVA and Sigma Plot Software, Jandel Scientific, Corte Madera, U.S.A.). After coronary occlusion, enhanced NADH-fluorescence was shown by an increase in the number of pixels with higher grey values. The number of pixels with enhanced NADH-fluorescence after coronary occlusion was proportional to the size of the ischaemic area.

The absolute size of the ischaemic zone was determined as the quotient of the NADH-fluorescence area 30 min after coronary occlusion and left ventricle size, as determined on epicardial NADH-fluorescence photographs.

Influence of the heart-rate on the left ventricular pressure and the coronary flow

Electrically-driven hearts ($n = 8$) were allowed to stabilize for 45 min before accelerating the pacing-rate stepwise by 30 beats min^{-1}, every 10 min from 180 beats min^{-1} up to 300 beats min^{-1}.

Concentration-response relationships

To investigate the haemodynamic effects of amrinone, milrinone or levosimendan, cumulative concentration-response curves were obtained in rabbit isolated spontaneously beating hearts. Hearts were allowed to stabilize for 45 min after preparation. Isoprenaline 10^{-10} M was added to the perfusion buffer to provide a β-adrenergic drive before applying amrinone ($n = 7$), milrinone ($n = 6$) or levosimendan ($n = 6$) at increasing concentrations at intervals of 10 min. Control hearts were examined to correct the haemodynamic parameters for changes occurring over time. The inotropic and coronary dilator activities were corrected for the influence of the heart-rate.

Influence of milrinone or levosimendan on acute regional ischaemia

Hearts were allowed to stabilize for 45 min after preparation before occluding a postero-lateral branch of the circumflex artery for 120 min. Milrinone (10^{-5} M, $n = 5$) or levosimendan-treatment (5×10^{-6} M, $n = 6$) was started after an ischaemic period of 30 min and continued over the experimental time. Untreated control hearts were examined for comparison ($n = 5$).

Epicardial NADH-fluorescence photographs were taken at $t = 30$ min, before starting the treatment, as well as at $t = 40$, 60, 90 and 120 min. The NADH-fluorescence area after an ischaemic period of 30 min was taken as a reference and set 100%.

Materials

The following substances were used: Amrinone (Sigma, St Louis, U.S.A.), milrinone (Sanofi-Winthrop, München, Germany), levosimendan ((−)-OR-125) ((**R**)-(−)-[[4-(1,4,5,6-tetrahydro-4- methyl-6-oxo-3- pyridazinyl) phenyl]-hydrazono] propanedinitril) (gift from Orion Pharmaceutica, Espoo, Finland), isoprenaline (Sigma, St Louis, U.S.A.).

Data analysis and statistics

Data are given as mean ± s.e.mean of n experiments. The relationship between heart-rate, left ventricular pressure and coronary flow was examined by linear regression analysis (Backhaus et al., 1994). Data from cumulative concentration-response curves were submitted to logit analysis to determine EC_{50} values (Hafner et al., 1977). Statistical significance was evaluated at 95% confidence limits by one way or two way ANOVA and Scheffée test.

Results

Influence of the heart-rate on the left ventricular pressure and the coronary flow

Accelerating the pacing-rate from 180 min^{-1} up to 300 min^{-1} was accompanied by a reduction of the left ventricular pressure (Δ LVP% = $-0.178 \times \Delta$ HR(%) + 1.248, linear correlation coefficient $r = -0.46$) and by an increase of the coronary flow (Δ CF(%) = $0.093 \times \Delta$ HR(%) + 2.301, $r = 0.54$).

Concentration-response relationships

The functional parameters did not differ significantly between the groups at the end of the equilibration period ($P > 0.05$) (Table 1), and they were not significantly affected by isoprenaline 10^{-10} M ($P > 0.05$). Amrinone, milrinone and levosimendan concentration-dependently increased the heart-rate (Figure 1), the left ventricular pressure (Figure 2) and the coronary flow (Figure 3). Milrinone showed a higher inotropic and coronary vasodilator potency than amrinone (Table 2), but the relation of inotropy to coronary dilatation was similar for both agents. There was also no significant difference between the maximal inotropic and coronary vasodilator effects of amrinone and milrinone ($P > 0.05$). The chronotropic potency was not determined as the maximal increase of the heart-rate could not be ascertained (Figure 1). Levosimendan showed a higher inotropic and coronary vasodilator potency than milrinone (Table 2). Whereas the maximal coronary vasodilatation was similar for the three agents ($P > 0.05$) (Figure 3), the maximal inotropic effect was significantly lower for levosimendan compared to amrinone or milrinone ($P < 0.05$) (Figure 2). Similarly, the maximal increase in heart-rate by levosimendan was significantly lower than that induced by amrinone or milrinone at the highest concentration used ($P < 0.05$) (Figure 1).

Table 1 Actual values of the heart-rate (HR), the left ventricular pressure (LVP) and the coronary flow (CF) at the end of the equilibration period before adding isoprenaline

	HR (min^{-1})	*LVP* (mmHg)	*CF* ($ml\ min^{-1}$)
Amrinone	117 ± 12	62 ± 8	18 ± 3
Milrinone	134 ± 13	59 ± 3	18 ± 2
Levosimendan	136 ± 5	61 ± 6	21 ± 1

There was no significant difference between the three groups ($P > 0.05$).

Table 2 EC_{50} values ($mol\ l^{-1}$) for the chronotropic, inotropic and coronary vasodilator activity of amrinone, milrinone and levosimendan

	Chronotropy	*Inotropy*	*Coronary dilatation*
Amrinone	–	2×10^{-5}	9.1×10^{-6}
Milrinone	–	7.7×10^{-6}	3×10^{-6}
Levosimendan	2.3×10^{-7}	7×10^{-7}	4.9×10^{-7}

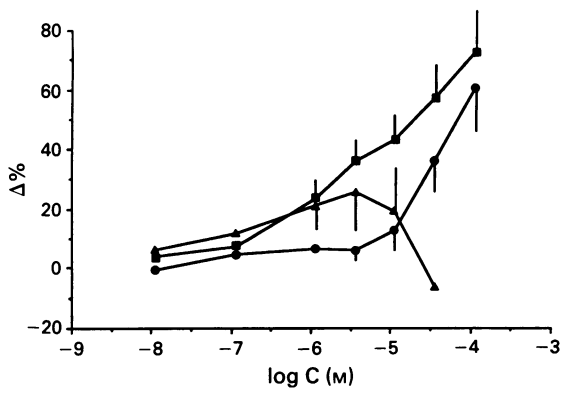

Figure 1 Cumulative concentration-response curves for the chronotropic effects of amrinone (●, $n = 7$), milrinone (■, $n = 6$) or levosimendan (▲, $n = 6$) in the presence of isoprenaline (10^{-10} M) in the perfusion buffer. Ordinate scale: increase in the heart-rate relative to the actual values before PDE-inhibitor application. Symbols represent the mean ± s.e.mean.

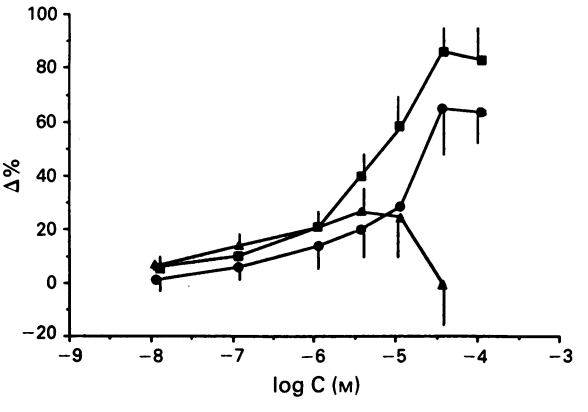

Figure 2 Cumulative concentration-response curves for the inotropic effects of amrinone (●, $n = 7$), milrinone (■, $n = 6$) or levosimendan (▲, $n = 6$) in the presence of isoprenaline (10^{-10} M) in the perfusion buffer. Ordinate scale: increase in the left ventricular pressure relative to the actual values before PDE-inhibitor application. Symbols represent the mean ± s.e.mean.

Effects of milrinone and levosimendan in regionally ischaemic hearts

There was no significant difference in pacing-rate, left ventricular pressure or coronary flow between treated hearts and controls at the end of the equilibration period when coronary occlusion was started ($P > 0.05$) (Table 3).

The coronary flow and concomitantly the left ventricular pressure were significantly decreased by coronary occlusion ($P < 0.05$) (Figures 4 and 5). The end-diastolic pressure was not significantly affected ($P > 0.05$) (data not shown). The left ventricular pressure and the pressure-rate-product were significantly increased to a similar extent by milrinone or levosimendan ($P < 0.05$) (Figure 5), whereas the end-diastolic pressure remained unaffected ($P > 0.05$) (data not shown). The coronary flow was enhanced by both agents ($P < 0.05$) (Figure 4) and the enhancement was similar for both agents over most of the time ($P > 0.05$) but was temporarily higher at $t = 40$ and 60 min in hearts treated with levosimendan ($P < 0.05$). Ventricular fibrillation did not occur in either group.

After coronary occlusion, myocardial ischaemia was visualized by a significant epicardial NADH-fluorescence enhancement distal to the occlusion site ($P < 0.05$). The size of the ischaemic zone (%) 30 min after coronary occlusion, prior to drug administration was not significantly different in the control (10 ± 3), milrinone-(11 ± 3) and levosimendan-(12 ± 3) treated hearts. In control hearts, myocardial ischaemia remained unchanged over the experimental time ($P > 0.05$) (Figure 6). Milrinone or levosimendan significantly reduced the epicardial NADH-fluorescence area ($P < 0.05$) (Figure 6). There was no significant difference between myocardial ischaemia reduction by milrinone or levosimendan ($P > 0.05$).

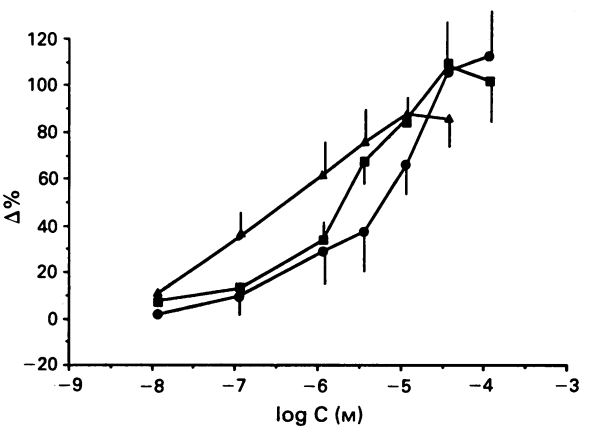

Figure 3 Cumulative concentration-response curves for the coronary vasodilator activity of amrinone (●, $n = 7$), milrinone (■, $n = 6$) or levosimendan (▲, $n = 6$) in the presence of isoprenaline (10^{-10} M) in the perfusion buffer. Ordinate scale: increase of the coronary flow relative to the actual values before PDE-inhibitor application. Symbols represent the mean ± s.e.mean.

Table 3 Actual values of the pacing-rate (HR), the left ventricular pressure (LVP) and the coronary flow (CF) at the end of the equilibration period, before coronary occlusion, and after an ischaemic period of 30 min, before starting the inotropic treatment

	HR (min^{-1})		*LVP* (mmHg)		*CF* ($ml\ min^{-1}$)	
Time (min)	0	30	0	30	0	30
Controls	202 ± 8		72 ± 4	61 ± 6	25 ± 3	18 ± 3
Milrinone	194 ± 5		65 ± 5	53 ± 5	24 ± 3	18 ± 3
Levosimendan	207 ± 10		68 ± 4	48 ± 6	24 ± 3	14 ± 2

There was no significant difference between the groups ($P > 0.05$).

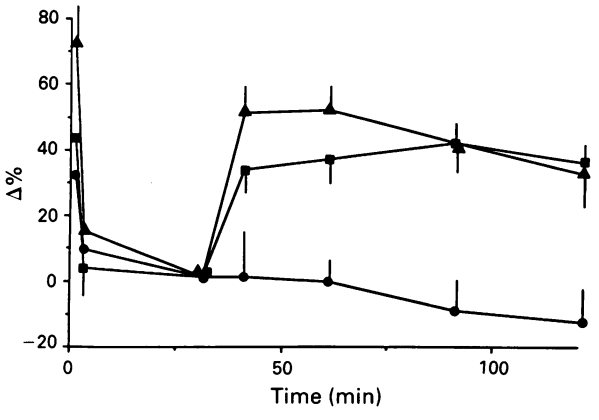

Figure 4 Time course of the relative changes of the coronary flow (CF) over the experimental time: (●) controls ($n = 5$); (■) milrinone 10^{-5} M ($n = 5$); (▲) levosimendan 5×10^{-6} M ($n = 6$). The actual values at $t = 30$ min, before starting the inotropic treatment, were taken as a reference (100%). Symbols represent the mean ± s.e.mean. CF enhancement was temporarily higher after levosimendan compared to milrinone-treatment at $t = 40$ and 60 min ($P < 0.05$).

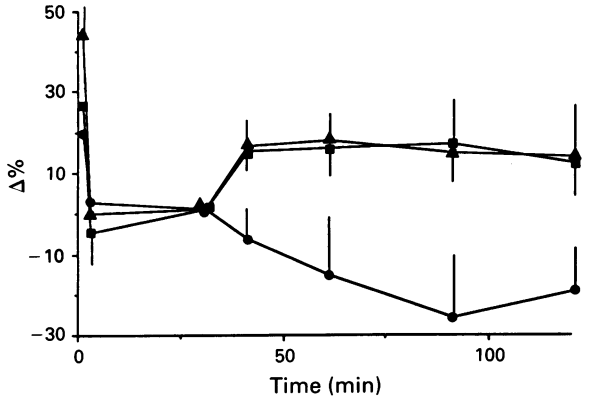

Figure 5 Time course of the relative changes of the left ventricular pressure (LVP) over the experimental time: (●) controls ($n = 5$); (■) milrinone 10^{-5} M ($n = 5$); (▲) levosimendan 5×10^{-6} M ($n = 6$). The actual values at $t = 30$ min, before starting the inotropic treatment, were taken as a reference (100%). Symbols represent the mean ± s.e. mean. There was no significant difference between LVP enhancement by milrinone and levosimendan ($P > 0.05$).

Discussion

The relation of inotropy to coronary vasodilatation was similar for the PDE-inhibitors, amrinone and milrinone. Although peripheral dilatation was not assessed in our experiments, these findings suggest that the haemodynamic profiles of both agents are very similar, and therefore similar anti-ischaemic potencies might also be expected. This is in accordance with previous findings showing that, at functionally equieffective concentrations, amrinone and milrinone

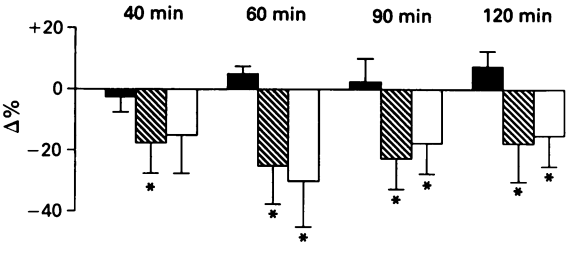

Figure 6 Epicardial NADH-fluorescence area over the experimental time. Filled columns: untreated control hearts ($n = 5$); hatched columns: milrinone-treated hearts ($n = 5$); open columns: levosimendan-treated hearts ($n = 6$). The fluorescence area 30 min after coronary occlusion was used as a reference (100%). Columns represent the mean ± s.e.mean, $*P < 0.05$.

diminished myocardial ischaemia and infarct size to a similar extent (Rump *et al.*, 1993a). Levosimendan showed a different functional profile with lower maximal effects on heart-rate and inotropy. The lower chronotropic and inotropic efficacy may be caused by an inhibitory action of levosimendan on the voltage-sensitive Ca^{2+} current (Raasmaja *et al.*, 1992), counteracting the chronotropic and inotropic effects of PDE-inhibition at higher concentrations. A decrease of the voltage-sensitive Ca^{2+} current in guinea-pig cardiomyocytes has been described for simendan at a concentration of 10^{-6} M, whereas twitch tension enhancement in guinea-pig papillary muscle shows an EC_{50} of 0.2×10^{-6} M (Raasmaja *et al.*, 1992). Calcium-channel blocking properties are also consistent with a marked coronary dilator activity, in spite of a lower maximal inotropic effect. Thus, a contribution of the calcium-antagonistic properties to cardioprotection, mediated by beneficial effects on the oxygen-demand/supply balance or by direct cytoprotection, must also be considered.

To assess the anti-ischaemic properties of milrinone and levosimendan, myocardial ischaemia was quantitated from epicardial NADH-fluorescence photography. This technique has been shown to be a very sensitive indicator of myocardial oxygenation (Barlow *et al.*, 1977). Moreover, tissue injury by toxic agents such as oxygen free radicals also induces an increase in NADH-fluorescence intensity (Rump *et al.*, 1993b,c). However, NAD redox state is measured only in the myoepicardium, which may not be characteristic of the myoendocardium. It was nevertheless demonstrated that a diminution of epicardial NADH-fluorescence during the experiment by pharmacological interventions is accompanied by infarct size reduction, as assessed by macrohistochemistry (Rump *et al.*, 1993a). Therefore, epicardial NADH-fluorescence seems to be a valid indicator of transmural ischaemic damage.

Myocardial oxygen-demand depends on heart-rate, wall tension and contractility (Baller *et al.*, 1979). The influence of the heart-rate can be negated in our model as hearts were paced at a constant rate. Furthermore, left ventricular pressure was measured isovolumetrically, excluding a reduction in wall-tension by ventricular geometry changes. The main determinants of myocardial oxygen consumption is therefore contractility and the only variable determinant of myocardial

oxygen-supply in our model is the global coronary flow. The anti-ischaemic effects of milrinone or levosimendan suggest an improvement of the oxygen-demand/supply balance. Several mechanisms must be considered: residual coronary flow to the ischaemic area is one of the most important determinants of the rate and extent of cell death within an ischaemic zone (Jennings & Reimer, 1983; Nienaber *et al.*, 1983; Hearse & Yellon, 1984). It was however demonstrated by NADH-surface fluorophotography (Harken *et al.*, 1981), anatomically (Flores *et al.*, 1984) and by microsphere techniques (Winkler *et al.*, 1984; Maxwell *et al.*, 1987) that collateral flow is essentially zero in rabbit hearts. Therefore, the likelihood of cardioprotection by drug-induced collateral flow enhancement seems remote. Concomitantly, direct cytoprotective effects may be largely excluded as the presence of the protective agent in the ischaemic tissue is required. As levosimendan was applied after the coronary occlusion was started, it may not have entered the ischaemic zone in substantial amounts. Calcium-channel blocking properties may re-inforce coronary vasodilatation, but a contribution to cardioprotection by inhibiting calcium-overload of cardiomyocytes in the ischaemic zone seems unlikely. It must rather be assumed that the beneficial effects of milrinone and levosimendan may be related to an increase of myocardial perfusion in the tissue surrounding the ischaemic zone, improving the oxygen-supply and metabolite elimination by diffusion exchanges. Moreover, as levosimendan and milrinone showed similar anti-ischaemic effects at equieffective concentrations, it seems that oxygen-sparing effects by sensitization of myofilaments to calcium do not contribute to the beneficial effects observed.

Although conclusions from *in vitro* experiments to a clinical setting must be drawn cautiously, our findings suggest that the bipyridine-type PDE-inhibitors amrinone and milrinone possess similar functional profiles, and therefore similar anti-ischaemic potencies might be expected. The anti-ischaemic properties of levosimendan are comparable to those of milrinone, at higher haemodynamically equieffective concentrations, associated with a marked coronary dilator activity. It remains to be seen whether oxygen-sparing effects, related to the calcium-sensitizing properties of levosimendan, confer to that compound particular cardioprotective properties at lower concentrations, when myocardial perfusion is less affected.

References

BACKHAUS, K., ERICHSON, B., PLINKE, W. & WEIBER, R. (1994). *Multivariate Analysenmethoden.* Berlin, Heidelberg, New York, London, Paris, Tokio, Hong Kong, Barcelona, Budapest: Springer Verlag.

BALLER, D., BRETSCHNEIDER, H.J. & HELLIGE, G. (1979). Validity of myocardial oxygen consumption parameters. *Clin. Cardiol.*, **2**, 317–327.

BARLOW, C.H., HARKEN, A.D. & CHANCE, B. (1977). Evaluation of cardiac ischemia by NADH fluorescence photography. *Ann. Surg.*, **186**, 737–740.

BENOTTI, J.R., GROSSMAN, W., BRAUNWALD, E. & CARABELLO, B.A. (1980). Effects of amrinone on myocardial energy metabolism and haemodynamics in patients with severe congestive heart failure due to coronary artery disease. *Circulation*, **62**, 24–34.

EDES, I., PAPP, G.J. & CSANADY, M. (1992). The effects of a new calcium sensitizer, simendan, on intracellular protein phosphorylation. *J. Mol. Cell. Cardiol.*, **24** (Suppl. V), 93.

FLORES, N.A., DAVIES, R.L., PENNY, W.J. & SHERIDAN, D.J. (1984). Coronary microangiography in the guinea pig, rabbit and ferret. *Int. J. Cardiol.*, **6**, 459–471.

HAFNER, D., HEINEN, E. & NOACK, E. (1977). Mathematical analysis of concentration-response relationships. *Arzneim.-Forsch./Drug Res.*, **27**, 1871–1873.

HAIKALA, H., LINDÉN, I.B. & POHTO, P. (1992a). Simendan, a novel calcium sensitizer, is as potent and efficient in the skinned fibre as in the electrically paced papillary muscle. *J. Mol. Cell. Cardiol.*, **24** (Suppl. I), 260.

HAIKALA, H., NISSINEN, E., ETEMADZADEH, E., LINDEN, I.B. & POHTO, P. (1992b). Levosimendan increases calcium sensitivity without enhancing myosin ATPase activity and impairing relaxation. *J. Mol. Cell. Cardiol.*, **24** (Suppl V), 97.

HARKEN, A.H., SIMSON, M.B., HASELGROVE, J., WETSTEIN, L., HARDEN, W.R. & BARLOW, C.H. (1981). Early ischemia after complete coronary ligation in the rabbit, dog, pig and monkey. *Am. J. Physiol.*, **241**, H202–H210.

HEARSE, D.J. & YELLON, D.M. (1984). Why are we still in doubt about infarct size limitation? The experimentalist's viewpoint. In *Therapeutic Approaches to Myocardial Infarct Size Limitation.* ed. Hearse, D.J. & Yellon, D.M. pp. 17–42. New York: Raven Press.

HERZIG, J.W. & QUAST, U. (1992). On the role of Ca^{++} binding proteins as possible targets for Ca^{++} sensitizing agents. *Z. Kardiol.*, **81** (Suppl. 4), 49–55.

HOLMBERG, S.R.M. & WILLIAMS, A.J. (1991). Phosphodiesterase inhibitors and the cardiac sarcoplasmic reticulum calcium release channel: differential effects of milrinone and enoximone. *Cardiovasc. Res.*, **25**, 537–545.

JENNINGS, R.B. & REIMAR, K.A. (1983). Factors involved in salvaging ischaemic myocardium: effect of reperfusion of arterial blood. *Circulation*, **68** (Suppl I), 125–136.

JENTZER, J.H., LEJEMTEL, T.H., SONNENBLICK, E.H. & KIRK, E.S. (1981). Beneficial effect of amrinone on myocardial oxygen consumption during acute left ventricular failure in dogs. *Am. J. Cardiol.*, **48**, 75–83.

KATZ, A.M. (1986). Potential deleterious effects of inotropic agents in the therapy of chronic heart failure. *Circulation*, **73** (Suppl. III), 184–190.

KITZEN, J.M. & WINBURY, M.M. (1989). Pimobendan. *Cardiovasc. Drug Res.*, **6**, 265–291.

LANGENDORFF, O. (1895). Untersuchungen am überlebenden Säugetier-herzen. *Arch. Ges. Physiol.*, **61**, 291–332.

LEYEN V DER, H., SCHMITZ, W., SCHOLZ, H. & SCHOLZ, J. (1989). New positive inotropic agents acting by phosphodiesterase inhibition or alpha1-adrenergic stimulation. *Pharmacol. Res.*, **21**, 329–337.

MAXWELL, M.P., HEARSE, D.J. & YELLON, D.M. (1987). Species variation in the corona collateral circulation during regional myocardial ischaemia: A critical determinant of the rate of evolution and extent of myocardial infarction. *Cardiovasc. Res.*, **21**, 737–746.

MORNER, S.E.J.N. (1990). Phosphodiesterase inhibitors increase calcium recirculation within the myocyte. In *Phosphodiesterase Inhibitors and the Heart.* pp. 6–7. Abingdon: The Medicine Group (UK) Ltd.

NIENABER, C., GOTTWICK, M., WINKLER, B. & SCHAPER, W. (1983). The relationship between perfusion deficit, infarct size and time after experimental coronary occlusion. *Basic Res. Cardiol.*, **78**, 210–226.

OVASKA, M., HAIKALA, H., LUNDSTROM, K., KORKOLAINEN, T., TASKINEN, J. & LINDÉN, I.B. (1992). Studies on calcium-dependent binding of simendan to troponin complex and troponin C. *J. Mol. Cell. Cardiol.*, **24** (Suppl. I), 260.

RAASMAJA, A., TALO, A., HAIKALA, H., NISSINEN, E., LINDÉN, I.B. & POHTO, P. (1992). In *Excitation-Contraction Coupling in Skeletal, Cardiac and Smooth Muscles.* ed. Frank, G.B., Bianchi, C.P. & Keuers, H.E.D. p. 423. New York, London: Plenum Press.

RUMP, A.F.E., BLAZINCIC, B. & KLAUS, W. (1993a). Effect of amrinone and milrinone on myocardial ischemia extent and infarct size in isolated rabbit hearts. *Drug Res.*, **43**, 1262–1266.

RUMP, A.F.E., RÖSEN, R. & KLAUS, W. (1993b). Cardioprotection by superoxide dismutase: A catecholamine-dependent process? *Anesth. Analg.*, **76**, 239–246.

RUMP, A.F.E., RÖSEN, R. & KLAUS, W. (1993c). Free radical scavenging properties of β-adrenoceptor blockers are not relevant for cardioprotection in isolated rabbit hearts. *Naunyn-Schmied. Arch. Pharmacol.*, **348**, 431–434.

RUMP, A.F.E., RÖSEN, R., KORTH, A. & KLAUS, W. (1993d). Deleterious effect of exogenous angiotensin-I on the extent of regional ischaemia and its inhibition by captopril. *Eur. Heart J.*, **14**, 106–112.

RUMP, A.F.E., RÖSEN, R., SIGMUND, B., FUCHS, J., DHEIN, S. & KLAUS, W. (1993e). Beneficial effect of amrinone on the size of acute regional ischemia in isolated rabbit hearts. *J. Cardiothor. Vasc. Anesth.*, **7**, 573–578.

VAN ZWIETEN, P.A. (1991). Background to the drug treatment of heart failure. In *The Role of Phosphodiesterase-Inhibitors in Heart Failure*. ed. Van Zwieten, P.A. pp. 1–12. London, New York: Royal Society of Medicine Services.

VENTURA, C., MILLER, R., WOLF, H.P., BEIER, N., JONAS, R., KLOCKOW, M., LUES, I., HANO, O., SPURGEON, H.A., LAKATTA, E.G. & CAPOGROSSI, M.C. (1992). Novel Diazinone Derivatives separate myofilament Ca^{2+} sensitization and phosphodiesterase III inhibitory effects in guinea pig myocardium. *Circ. Res.*, **70**, 1081–1090.

WINKLER, B., SASS, S., BINZ, K. & SCHAPER, W. (1984). Myocardial blood flow and myocardial infarction in rats, guinea pig and rabbits. *J. Mol. Cell. Cardiol.*, **16** (Supp 2), 48.

(Received July 1, 1993
Revised February 24, 1994
Accepted March 9, 1994)