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Effect of prostaglandin I_2 analogues on left ventricular diastolic function in vivo

Hille Kisch-Wedel [a,b,*], Gregor Kemming [a,b], Franz Meisner [b,d], Michael Flondor [a,c], Sebastian Bruhn [b], Carolina Koehler [b], Konrad Messmer [b], Bernhard Zwissler [c]

[a] *Clinic of Anesthesiology, Ludwig Maximilian University, Marchioninistr. 15, D-81377 Munich, Germany*
[b] *Institute for Surgical Research, Ludwig Maximilian University, Marchioninistr. 27, D-81377 Munich, Germany*
[c] *Clinic for Anesthesiology, Intensive Care Medicine and Pain Therapy of the Johann Wolfgang Goethe University, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany*
[d] *Department of Thoracic and Vascular Surgery, Surgical Clinic, University of Ulm, Germany*

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Abstract

The prostaglandin I_2 analogues epoprostenol and iloprost increase left ventricular contractility. Therefore, we hypothesize that the prostaglandin I_2 analogues epoprostenol and iloprost improve also left ventricular diastolic function. To test this hypothesis, the effects of epoprostenol and iloprost on left ventricular diastolic function were assessed in vivo and compared to two vasodilators sodium nitroprusside and adenosine, not formerly associated with changes of left ventricular contractility. Eleven pigs (25.9±2.8 kg, balanced anaesthesia) were exposed to the short-acting intravenous vasodilators sodium nitroprusside, adenosine and epoprostenol in a randomized cross over design. The long-acting iloprost was administered at the end of the protocol. The drugs are titrated to achieve a 25% reduction of diastolic aortic pressure. Active isovolumic relaxation properties of the left ventricle were assessed by the maximum velocity of left ventricular pressure drop. Passive phase of relaxation and filling was assessed by the determination of end diastolic compliance during a preload reduction manoeuvre.

The maximum velocity of left ventricular pressure drop worsened during the infusion of sodium nitroprusside (baseline: −1950; sodium nitroprusside: −1293 mm Hg/s, $p<0.05$, Wilcoxon signed rank test versus vs. baseline) and adenosine (baseline: −2015; adenosine: −1345 mm Hg/s, $p<0.05$), but remained stable during the infusion of the prostaglandins (baseline: −1943; epoprostenol: −1785 mm Hg/s; baseline: −2042; iloprost: −1923 mm Hg/s). End diastolic compliance was not altered significantly by any vasodilator. Interstitial myocardial cAMP increased during the infusion of epoprostenol (7.60 to 13.87 fmol/ml, $p<0.05$) and tended to increase during the infusion of iloprost (7.56 to 11.66 fmol/ml, $p=0.21$). The prostaglandin I_2 analogues epoprostenol and iloprost preserved the early phase of active isovolumic relaxation, presumably mediated by myocardial cAMP, whereas sodium nitroprusside and adenosine impaired early active isovolumic relaxation. Passive relaxation and filling properties remained stable during the infusion of each applied vasodilator in the intact left ventricle in vivo.

Keywords: Diastole; Prostaglandins; Vasodilatation; Adenosine; Nitric oxide

1. Introduction

Vasodilator therapy is a common concept for the treatment of *heart failure* to optimize left heart function (Taylor et al., 2004) or to treat pulmonary hypertension to improve right heart function by pre- and afterload reduction (Zwissler, 2000). Yet, a common side effect is

* Corresponding author. Clinic for Anesthesiology, Ludwig-Maximilians University, Munich, Marchioninistr. 15, D-81377, Munich, Germany. Tel.: +49 89 7095 789 1145.
E-mail address: Hille.Kisch-Wedel@med.uni-muenchen.de (H. Kisch-Wedel).

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tachycardia caused by the vasodilatation induced baroceptor reflex (Macov et al., 1992). Indeed, tachycardia mainly reduces the duration of diastole and to a smaller extent the duration of systole. During the infusion of various vasodilators the reduction in diastolic duration may impair diastolic function to a different extent in vivo. To test this hypothesis, the action of the prostacyclin analogues epoprostenol and iloprost, the nitric oxide donor sodium nitroprusside, and adenosine on left ventricular diastolic function were compared in an experimental study in vivo.

2. Materials and methods

2.1. Anaesthesia

The study was performed in 11 German house pigs (mean body weight 25.9±2.8 kg) after approval of the government of upper Bavaria (no. AZ 76/99) for the study in animal subjects. All animals received care in compliance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23, revised 1985). They were premedicated with 10 mg/kg body weight ketamine and 1 mg/kg midazolam intramuscularly. Anaesthesia was induced intravenously with 0.02 mg/kg fentanyl, 1.5–2 mg/kg propofol and muscle relaxation was achieved with 0.4 mg/kg vecuronium bromide. After intubation the animals were ventilated in a volume controlled mode (p_aCO_2 35–40 torr) with 50% O_2, 47–46% N_2O, and isoflurane 2–3 vol% (Servo 900B, Siemens-Elema, Solna, Sweden). Fentanyl (0.04 mg/kg/h), and vecuronium bromide (0.4 mg/kg/h) were continuously infused. A warming pad was used, and mean core body temperature was kept at 38.5±0.6 °C.

2.2. Surgical preparation and placement of catheters

Animals were tracheotomized, the left carotid artery, right and left external jugular vein, right and left femoral artery and vein were dissected and cannulated. A subxiphoidal access was chosen to place a microdialysis probe (with 20 kD cut off-membrane; CMA/Microdialysis, Solna, Sweden). The pericardium was opened for 3–5 cm and the probes were inserted in the left anterior myocardium to determine interstitial left myocardial cyclic adenosine (cAMP). One animal died due to ventricular fibrillation during placement of the left myocardial microdialysis probe. The following catheters were placed:

1) A 7F 12 electrode conductance pressure catheter (with pigtail, dual field, electrode spacing 8 or 10 mm, Cardiodynamics, Leyden, Netherlands): left ventricle.
2) A 22F Fogarty-catheter (Baxter, Irvine, CA, USA): inferior caval vein.
3) An electronic tip manometer (PC 370, Millar instruments, Houston, Texas, USA): abdominal aorta.
4) A right ventricular ejection fraction/volumetric catheter (REF-1™, Baxter, Irvine, California, USA) for determination of cardiac index: pulmonary artery.
5) A 12G triple lumen large bore catheter (Arrow, Reading, USA): left femoral vein.
6) A 8.5F introducer sheath (Arrow, Reading, USA): left femoral artery.

Correct catheter position was verified by fluoroscopy and by evaluation of volume and pressure signals.

2.3. Methods of measurement

2.3.1. Left ventricular diastolic function

The *duration of systole* was defined as the time between start of the heart cycle until peak ejection, which is the minimum of the first derivative of the left ventricular volume over time. The duration of the diastole was defined as the diastolic filling time, which is the time from the onset of maximum velocity of left ventricular pressure drop until the end-diastole. Both parameters were automatically derived with a sample rate of 4 ms by means of the conductance method (Conduct-PC, Version V720.1, Cardiodynamics, Leyden, Netherlands).

The *early active phase of isovolumic relaxation* (Brutsaert and Sys, 1989) was assessed by the maximum velocity of left ventricular pressure drop. A faster drop of maximum velocity of left ventricular pressure drop describes positive lusitropism, and a slower drop of maximum velocity of left ventricular pressure drop is negative lusitropism. The *late phase of active isovolumic relaxation* was measured by quantifying the time constant τ, which was calculated from the time of the maximum velocity of left ventricular pressure drop until pressure reaches half the value at the maximum velocity of left ventricular pressure drop according to Mirsky (1984). When τ increases or prolongs, late active isovolumic relaxation impairs, while a decrease of τ indicates a faster or better late active isovolumic relaxation. The maximum velocity of left ventricular pressure drop and τ were digitally assessed by a commercially available computer program with a sample rate of 4 ms (Conduct-PC, Version V720.1, Cardiodynamics, Leyden, Netherlands). Due to the fact that the heart rate has not been kept constant in the present study we calculated the relative τ, the ratio of τ to the duration of a single heart cycle (τ_r). When the maximum velocity of left ventricular pressure drop increases, the speed of relaxation is reduced and, therefore, early active isovolumic relaxation deteriorates.

The *slope of the end diastolic pressure volume relation*, the left ventricular end diastolic compliance, was determined as a measure of the *late passive phase of relaxation and filling* of the left ventricle (Schertel, 1998). The left ventricular volume signal was calculated from the conductance signal (see Eq. (1)). At each baseline measurement, 5 ml hypertonic sodium chloride (20%) was injected to assess parallel conductance at a period of suspended ventilation. Estimation of parallel conductance is described in detail elsewhere (Baan et al., 1984). Reduction of preload was achieved by inflating the Fogarty catheter in the inferior caval vein with 5–10 ml of 0.9% saline solution during a period of suspended ventilation. Pressure and volume were registered digitally with a sample time of 4 ms. The end diastolic volume and pressure data during the preload reduction manoeuvre were registered by a computer program (Conduct-PC, Version V720.1, Cardiodynamics, Leyden, Netherlands). After exclusion of the first three beats (change of parallel conductance by the filled right ventricle) and a constant decrease in pressure and volume during the preload reduction manoeuvre data were included for further analyses: A regression line was fitted through

the end diastolic volume (x) and pressure points (y) and the *slope* of this regression line was determined, which is the *end diastolic compliance.* An example is depicted in Fig. 2. A high mean correlation coefficient of 0.94 resulted for all data. The end diastolic compliance of the left ventricle is a global, mainly load-independent estimate of the late passive phase of left ventricular relaxation and filling (Baan et al., 1984). When the end diastolic compliance is high, the *slope* of the end diastolic pressure volume relation is steep and might be described as "stiff ventricle". In contrast, a "compliant ventricle" is filled with big volumes at a small pressure gradient resulting in a *low slope* of the end diastolic pressure volume relation, a small end diastolic compliance.

2.3.2. Left ventricular interstitial cAMP

The microdialysis probe in the left myocardium was perfused with Krebs Ringer solution 3 μl/min and interstitial probes were collected by a micro fraction collector every 10 min (CMA 140 CMA/Microdialysis, Solna, Sweden). Samples were frozen at −20 °C. Interstitial myocardial cAMP (cAMP) was estimated by a commercially available enzyme linked immunoadsorbant assay (ELISA) (R&D systems, Wiesbaden-Nordenstadt, Germany). Optical densities were measured in an ELISA-reader at 405 nm (Dynex Revelation G 3.2. Sullyfield Circle, Chantilly, Virginia, USA) with a reference at 570 nm and concentrations were calculated. (see Eq. (5)).

2.3.3. Calculation of parameters

Eq. (1): Left ventricular volume at time t ($V(t)$) (Baan et al., 1984)

$$V(t) = \rho^{*}L^{2}{}^{*}\sum G_n(t) - \rho^{*}L^{2}\ G_p, \tag{1}$$

where $V(t)$: Volume at time t; ρ: Calibration factor for blood conductivity; Gn(t): Conductance of one catheter segment at time t, where $n=\{1,\ldots,4,5\}$ including only segments of the catheter which were placed in the left ventricle as indicated by segmental signals; Gp: Parallel conductance of the surrounding tissue.

The conductance signal was calibrated to blood conductivity in a "rho cuvette" (Cardiodynamics, Leyden, Netherlands) adapted to the Leycom Sigma-5DF signal conditioner processor (Cardiodynamics, Leyden, Netherlands) immediately before each measurement. Another calibration factor α, which is generally used for calculation of $V(t)$ was set to 1. End systolic elasticity is therefore not influenced by the variability of another method for cardiac output determination (for example by thermo dilution).

Eq. (2): cardiac index (CI)

$$CI = CO/BSA, \tag{2}$$

where BSA is body surface area, CO the cardiac output.

Eq. (3): systemic vascular resistance index (SVRI)

$$SVRI = (MAP - CVP)/CI^{*}c, \tag{3}$$

where $c=79.9$ (conversion factor), MAP is the mean aortic blood pressure, CVP the central venous pressure and CI the cardiac index.

Eq. (4): pulmonary vascular resistance index (PVRI)

$$PVRI = (MPAP - PCWP)/CI^{*}c, \tag{4}$$

where $c=79.9$ (conversion factor), MPAP is the mean pulmonary artery pressure, PCWP the pulmonary artery wedge pressure and CI the cardiac index.

Eq. (5): calibration curve for left myocardial interstitial cAMP concentration (cAMP)

$$cAMP(\mathrm{fmol/ml}) = 1086.4^{*}e^{-21.67^{*}ODcAMP}\ \left(R^2 = 0.936\right), \tag{5}$$

where ODcAMP is the optical density at 20, 5, 1.25, 0.312, 0.78, 0.0195, 0.0050 pmol/ml cAMP standard concentrations.

2.4. Experimental protocol

After a baseline measurement vasodilators were titrated by continuous infusion to achieve a stable reduction (for 35 min in median) of diastolic aortic pressure to 75% of the baseline value and then a second measurement was performed. Thereafter, vasodilators were reduced stepwise to avoid rebound hypertension followed by a washout period of at least 30 min. A subsequent baseline measurement was then performed. This resulted in a measurement before, during and after application of the short-acting substances. Adenosine (adenosine free base, ICN Biomedicals Inc., Aurora, Ohio, USA), epoprostenol (epoprostenol, Flolan®, Glaxo Wellcome, Versaille, France) and sodium nitroprusside (sodium nitroprusside, nipruss®, SCHWARZ PHARMA AG, Monheim, Germany) were randomized in a crossover design. Iloprost (iloprost trometamol, Ilomedin® Schering, Berlin, Germany) was always infused at the end of the protocol without a measurement after iloprost application due to its longer half life time. Each animal received all substances according to the cross over design of the study.

2.5. Statistical analysis

Data are presented as median ± semi-interquartile range ((Q_1–Q_3)/2) or as each single experiment. Individual differences were calculated: 1) between substance application and before; 2) after and before substance application. These data sets were tested by the Wilcoxon signed rank test. An α-error lower than 0.05 was considered to be statistically significant. The comparison between vasodilators (for the maximum velocity of left ventricular pressure drop) was performed by Friedman repeated measures analysis of variance on ranks followed by a Student–Newman–Keuls pair wise multiple comparison procedure.

3. Results

Diastolic aortic pressure was reduced to comparable values by adjusting the dose of the vasodilators. After stepwise withdrawal of sodium nitroprusside, adenosine and epoprostenol diastolic aortic pressure returned to baseline values. Compared to baseline sodium nitroprusside reduced diastolic aortic pressure to 74%, adenosine to 69%, epoprostenol to 73% and iloprost to 70%, respectively.

3.1. Electrolytes

Sodium concentrations slightly increased during and after infusion of each vasodilator ($p<0.05$, Tables 1-4), but stayed

Table 1
Effects of intravenous adenosine

		Before	Adenosine	After
Hemodynamics and respiratory data	Equipotent dose (μg/kg/min)		106±35	
	Diastolic arterial pressure (mm Hg)	62±10	43±6[a]	57±11
	Heart rate (beats/min)	124±16	126±19	126±19[a]
	Pulmonal arterial pressure (mm Hg)	25±3	26±3	27±2[a]
	Cardiac index (l/min/m^2)	4.5±0.8	5.4±0.8[a]	5.2±0.9[a]
	Systemic vascular resistance index (dyne s/cm^5/m^2)	2208±834	1491±431[a]	1867±759[a]
	Pulmonal vascular resistance index (dyne s/cm^5/m^2)	543±189	421±164	334±158
	Arterial O_2-partial pressure (torr)	187±35	137±62[a]	182±52
	Arterial CO_2-partial pressure (torr)	40±5	41±4	40±3
Left ventricular diastolic function	Constant of relaxation tau (ms)	26±3	25±1	23±3[a]
	Diastolic filling time (ms)	218±29	208±39	184±43[a]
	LV enddiastolic pressure (mm Hg)	10±2	10±2	12±2
	LV enddiastolic volume (ml)	44±7	45±6	47±6
	LV endsystolic volume (ml)	24±8	26±6	26±7
	Peak filling rate (ml/s)	186±41	197±26	191±32
Electrolytes	Sodium (mmol/l)	136±2.2	138±1.9[a]	140±1.7[a]
	Potassium (mmol/l)	4.4±0.1	4.4±0.2	4.4±0.3
	Calcium (mmol/l)	1.18±0.05	1.21±0.06	1.21±0.05

Data are presented as median±semi-interquartile range; [a] $p<0.05$ versus before.

Table 2
Effects of intravenous sodium nitroprusside

		Before	Sodium nitroprusside	After
Hemodynamics and respiratory data	Equipotent dose (μg/kg/min)		9.7±2.5	
	Diastolic arterial pressure (mm Hg)	64.8±12	48±5[a]	61±6
	Heart rate (beats/min)	125±9	164±23[a]	111±18
	Pulmonal arterial pressure (mm Hg)	26±3	22±2[a]	27±2
	Cardiac index (l/min/m2)	4.8±0.5	4.6±0.5	4.6±0.8
	Systemic vascular resistance index (dyne s/cm^5/m^2)	1867±625	1729±594[a]	2179±404
	Pulmonal vascular resistance index (dyne s/cm^5/m^2)	478±176	358±135	456±127
	Arterial O_2-partial pressure (torr)	164±52	146±40	159±38
	Arterial CO_2-partial pressure (torr)	41±3	41±3	40±2
Left ventricular diastolic function	Constant of relaxation tau (ms)	25±2	22±2[a]	25±1
	Diastolic filling time (ms)	227±49	149±27[a]	228±46
	LV enddiastolic pressure (mm Hg)	10±3	6±2[a]	11±2
	LV enddiastolic volume (ml)	45±6	34±4	47±13
	LV endsystolic volume (ml)	26±6	29±4	33±12
	Peak filling rate (ml/s)	191±32	183±43	195±38
Electrolytes	Sodium (mmol/l)	138±2.5	139±1.7[a]	139±1.6
	Potassium (mmol/l)	4.5±0.2	4.7±0.3	4.6±0.3
	Calcium (mmol/l)	1.22±0.07	1.21±0.05	1.21±0.02

Data are presented as median±semi-interquartile range; [a] $p<0.05$ versus before.

Table 3
Effects of intravenous epoprostenol

		Before	Epoprostenol	After
Hemodynamics and respiratory data	Equipotent dose (μg/kg/min)		0.18±0.09	
	Diastolic arterial pressure (mm Hg)	62±5	45±5[a]	61±9
	Heart rate (beats/min)	107±21	155±21[a]	125±15
	Pulmonal arterial pressure (mm Hg)	27±4	23±2	25±2
	Cardiac index (l/min/m^2)	4.4±0.9	5.5±1.2[a]	4.8±1.3[a]
	Systemic vascular resistance index (dyne s/cm^5/m^2)	2402±785	1222±339[a]	2026±593[a]
	Pulmonal vascular resistance index (dyne s/cm^5/m^2)	455±180	362±132[a]	394±181[a]
	Arterial O_2-partial pressure (torr)	177±32	153±21	193±27
	Arterial CO_2-partial pressure (torr)	40±2	39±2	40±4
Left ventricular diastolic function	Constant of relaxation tau (ms)	25±5	22±2[a]	24±2
	Diastolic filling time (ms)	228±64	154±35[a]	206±47
	LV enddiastolic pressure (mm Hg)	10±3	9±1	13±3
	LV enddiastolic volume (ml)	51±14	41±14[a]	47±7
	LV endsystolic volume (ml)	34±13	26±11[a]	24±8
	Peak filling rate (ml/s)	186±55	212±33	212±53
Electrolytes	Sodium (mmol/l)	137±2.4	138±2.4[a]	138±2.1[a]
	Potassium (mmol/l)	4.6±0.3	4.6±0.1	4.4±0.2[a]
	Calcium (mmol/l)	1.22±0.03	1.21±0.02	1.18±0.04[a]

Data are presented as median±semi-interquartile range; [a] $p<0.05$ versus before.

within normal ranges. Potassium and calcium levels were stable. A slight decrease in potassium level occurred after infusion of epoprostenol ($p<0.05$, Table 3).

3.1.1. Duration of systole and diastole

Tachycardia subsequent to the infusion of vasodilators significantly reduced the diastolic time interval (diastolic time interval, Tables 1-4, $p<0.05$, Fig. 1), but did only slightly reduce the systolic time interval until peak ejection rate (Fig. 1). The systolic time interval was significantly reduced during the infusion of epoprostenol and iloprost.

3.1.2. Left ventricular diastolic function:

Left ventricular diastolic function is divided into two major phases as depicted in a loop in Fig. 2: Isovolumic relaxation and left ventricular filling.

Early active phase of isovolumic relaxation: The maximum velocity of left ventricular pressure drop decreased during the infusion of sodium nitroprusside and adenosine ($p<0.05$, Fig. 3A), but remained unchanged during the prostaglandin analogues epoprostenol and iloprost. The maximum velocity of left ventricular pressure drop did not correlate with heart rate (Fig. 3B, $R^2=0.0013$, $p=0.737$).

Table 4
Effects of intravenous iloprost

		Before	Iloprost
Hemodynamics and respiratory data	Equipotent dose (μg/kg/min)		0.28±0.03
	Diastolic arterial pressure (mm Hg)	56±7	40±8[a]
	Heart rate (beats/min)	125±20	164±24[a]
	Pulmonal arterial pressure (mm Hg)	26±2	23±2[a]
	Cardiac index (l/min/m^2)	5.2±1.2	6.1±0.6[a]
	Systemic vascular resistance index (dyne s/cm^5/m^2)	1828±406	1250±277[a]
	Pulmonal vascular resistance index (dyne s/cm^5/m^2)	338±145	295±110[a]
	Arterial O_2-partial pressure (torr)	182±28	142±37[a]
	Arterial CO_2-partial pressure (torr)	37±3	39±3
Left ventricular diastolic function	Constant of relaxation tau (ms)	24±2	21±1[a]
	Diastolic filling time (ms)	204±44	151±20[a]
	LV enddiastolic pressure (mm Hg)	13±2	8±1[a]
	LV enddiastolic volume (ml)	47±7	44±10
	LV endsystolic volume (ml)	33±9	29±9
	Peak filling rate (ml/s)	202±41	218±20
Electrolytes	Sodium (mmol/l)	140±2.6	142±2.7[a]
	Potassium (mmol/l)	4.3±0.3	4.4±0.2
	Calcium (mmol/l)	1.2±0.03	1.19±0.03

Data are presented as median±semi-interquartile range; [a] $p<0.05$ versus before.

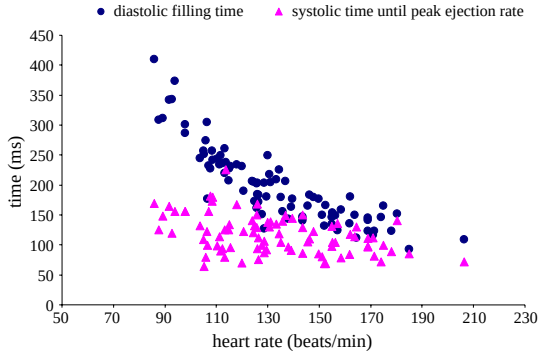


Fig. 1. The influence of heart rate on the duration of diastole and systole: while diastolic filling time (dots) is notably reduced with increasing heart rate, tachycardia did only slightly affect the duration of the systole until peak ejection rate (triangles, all data $n=86$).

Late active phase of isovolumic relaxation: τ shortened during the infusion of sodium nitroprusside, epoprostenol and iloprost ($p<0.05$, Tables 2-4). In contrast, the ratio of τ to a single heart beat, $\tau_{r,}$ increased during the infusion of sodium nitroprusside, epoprostenol and iloprost when tachycardia was present ($p<0.05$, Fig. 4A,B), indicating that the late phase of active isovolumic relaxation needed relatively more time within a single heart beat. Both, heart rate and τ_r remained unchanged during the infusion of adenosine. After withdrawal of adenosine a slight decrease of τ occurred ($p<0.05$, Table 1).

Passive phase of relaxation and filling: Looking at all data neither sodium nitroprusside or adenosine nor the prostaglandin analogues epoprostenol and iloprost altered the end diastolic compliance (Fig. 5) and the peak filling rate (Tables 1-4) significantly.

3.2. Left ventricular interstitial cAMP

Infusion of prostaglandin I_2 analogues reversibly increased left myocardial interstitial cAMP ($p<0.05$, Fig. 6). There was a trend for iloprost to increase the level of left myocardial interstitial cAMP ($p=0.21$, Fig. 6).

4. Discussion

4.1. Main findings

The first main finding of the present study is that adenosine and sodium nitroprusside slowed the maximal speed of early active isovolumic relaxation (the maximum velocity of left ventricular pressure drop). In contrast, the maximum velocity of left ventricular pressure drop remained unchanged during the infusion of the prostaglandin analogues epoprostenol and iloprost. There was also a tendency of the peak filling rate to increase during the infusion of the prostaglandin analogues. This preservation of early active relaxation during the infusion of the prostaglandins, especially during epoprostenol, is probably due to the elevation of the second messenger cAMP in the interstitial myocardium (Fig. 6). Due to the fact that cAMP is released from myocardial cells, we conclude that interstitial cAMP is directly related to intramyocardial cAMP (O'Brian and Strange, 1975). Further more Müller et al. have shown that the positive inotropic acting phosphodiesterase inhibitor milrinone dose dependently increased interstitial cAMP of skeletal muscle and milrinone is known to elevate intramyocardial cAMP by inhibition of phosphodiesterase 3 activity. An increase in intramyocardial cAMP is generally known either to be caused by a receptor dependent stimulation of adenylate cyclase enhancing cAMP production or by an inhibition of phosphodiesterase slowing cAMP degradation. The first possible mechanism of a cAMP mediated faster relaxation is known for β-agonists: β-agonists increase myocardial cAMP thereby regulating the phosphorylation of troponin I and reducing the interaction between troponin C and calcium ions (Parker et al., 1991). Then, the actin–myosin bridges are solved easier and relaxation is faster (the maximum velocity of left ventricular pressure drop decreases). The second possible mechanism is that the calcium ion current from the cytoplasm of the myocardial cell into the sarcoplasmatic reticulum increases cAMP thereby leading to a faster relaxation (Parker et al., 1991).

The second main finding of the study is that vasodilator induced tachycardia did not impair left ventricular end diastolic function of the intact left ventricle despite a reduced diastolic filling time (Fig. 1). Tachycardia subsequent to the infusion of vasodilators only slightly reduced the systolic time interval, but considerably reduced diastolic time interval (Fig. 1). The systolic time interval is linearly related to heart rate, whereas the diastolic time interval exponentially decreases with increasing heart rate. This nonlinear relationship of diastolic time to heart rate was also described for various other pharmacologic agents (Boudoulas et al., 1979).

Our data suggest that 1) epoprostenol and iloprost preserved early active diastolic relaxation presumably mediated by an increase in myocardial cAMP, thereby maintaining global end diastolic function in the intact

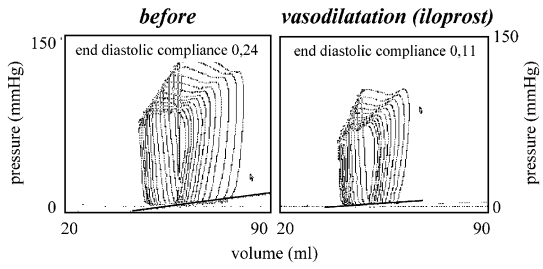


Fig. 2. Left ventricular pressure volume loops before and during vasodilatation with iloprost from a single experiment: during vasodilatation with iloprost the loops were smaller and slightly shifted to the left side, the preload reduction manoeuvre (inflation of the Fogarty catheter in the inferior caval vein) further shifts the loops to the left and they become smaller. A regression line is fitted through the end diastolic points. The slope of this line is the end diastolic compliance. In this particular experiment end diastolic compliance is 0.24 (mm Hg/ml) at control and drops (i.e. improves to 0.11 mm Hg/ml) during infusion of iloprost.

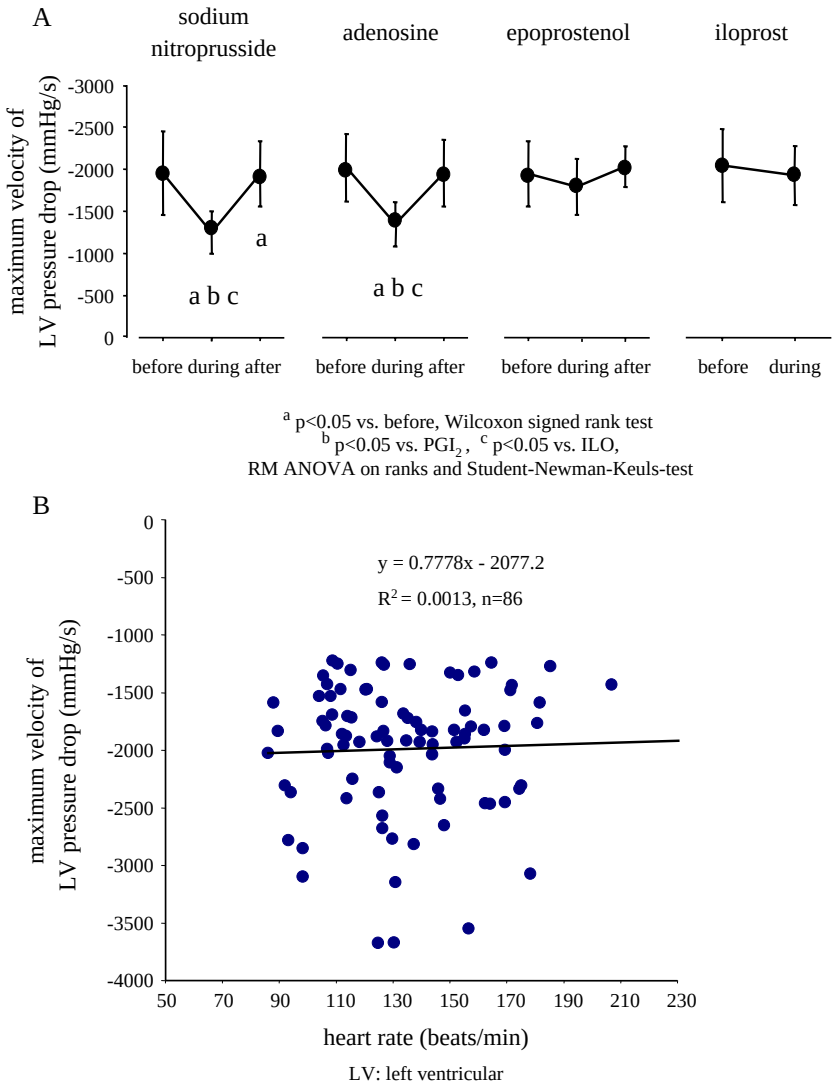


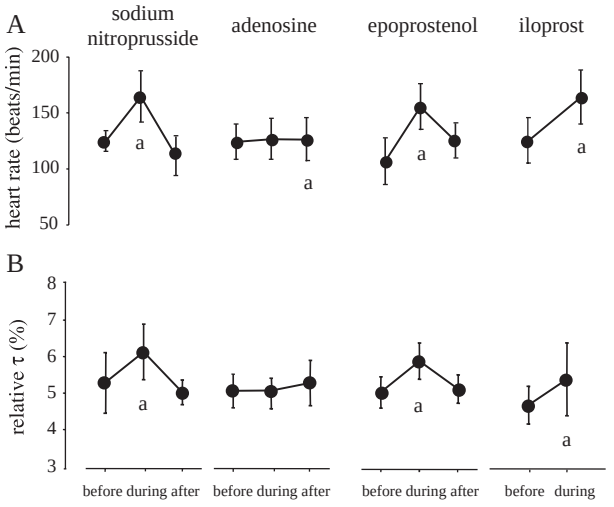
Fig. 3. The maximum velocity of left ventricular (LV) pressure drop worsened during the infusion of sodium nitroprusside and adenosine, whereas the maximum velocity of left ventricular pressure drop remained stable during the infusion of the prostaglandin I_2 analogues epoprostenol and iloprost (Fig. 3A). The maximum velocity of left ventricular (LV) pressure drop did not correlate with heart rate (Fig. 3B).

myocardium in vivo, and that 2) adenosine and sodium nitroprusside exert a negative lusitropic effect, i.e. slowed down the speed of early active relaxation, while preserving global end diastolic function in the intact myocardium as estimated by the end diastolic compliance.

4.2. Effects of different vasodilators on left diastolic ventricular function

In patients with congestive heart failure the inodilator milrinone improved diastolic performance: milrinone increased the maximum velocity of left ventricular pressure drop, and decreased (i.e. improved) the time constants of left ventricular relaxation. Consecutively, the peak filling rate of the left ventricle increased (Monrad et al., 1984). A similar improvement of isovolumic relaxation, an increase in the maximum velocity of left ventricular pressure drop and a decrease in the time constant τ was shown during β-stimulation by the infusion of dobutamine in patients with congestive heart failure, when heart rate was kept constant (Parker et al., 1991).

The underlying cause for the improvement of diastolic function by milrinone, a phosphodiesterase 3 inhibitor, and dobutamine, a β_1-agonist, could be the intracellular rise in cAMP in the myocardium. The data of the present study suggest that prostaglandin I_2 exerts its effects via the formation of cAMP. Epoprostenol caused a reversible rise of cAMP in the interstitium of the left myocardium (microdialysis), iloprost at least tended to increase cAMP (Fig. 6). The positive inotropic effect of milrinone is mediated by an *intracellular* rise of cAMP, and a rise of cAMP in the *interstitium* of skeletal muscle by milrinone was shown (Müller et al., 1997). A substantial positive inotropic effect has been recently shown for epoprostenol and iloprost (Kisch-Wedel et al., 2003). As the late systole is coupled to the early diastole, positive inotropism could lead



[a] p<0.05 vs. before, Wilcoxon signed rank test

Fig. 4. Heart rate increased during the infusion of sodium nitroprusside, epoprostenol and iloprost. A slight increase of heart rate occurred after withdrawal of adenosine (Fig. 4A). The relative τ is the fraction of τ from one total heart beat (%). Relative constant τ of isovolumic relaxation increased during the infusion of sodium nitroprusside, epoprostenol and iloprost (Fig. 4B) when tachycardia was present. During the infusion of adenosine relative τ remained constant when heart rate was stable.

to positive lusitropism. *Early active relaxation*: in particular cAMP-dependent protein kinases phosphorylate the protein phospholamban. The phosphorylation of phospholamban abolishes the inhibitory effects of phospholamban on calcium ATP-ase in the sarcoplasmatic reticulum of the cardiac myocyte. The calcium uptake from the cytosol into the sarcoplasmatic reticulum is thereby accelerated and the consecutive rapid decline in cytosolic calcium concentration facilitates and speeds up cardiac relaxation, i.e. acts positive lusitropic (Gillebert et al., 1989; Wallace et al., 1995).

The decrease in cytosolic calcium level is the driving force for early active relaxation, which was assessed by means of the maximal velocity of pressure drop. The maximum velocity of left ventricular pressure drop was preserved by the infusion of epoprostenol and iloprost, whereas it deteriorated during the infusion of adenosine and sodium nitroprusside indicating an increased early diastolic myocardial stiffness. The maximum velocity of left ventricular pressure drop failed to correlate with heart rate (Fig. 3B, $R^2=0.0013$, $p=0.737$). This suggests, that tachycardia is unlikely to be the mechanism preserving or increasing the maximum velocity of left ventricular pressure drop.

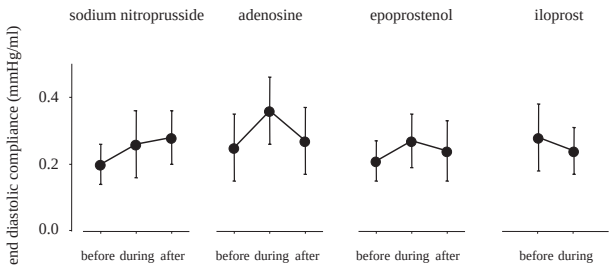
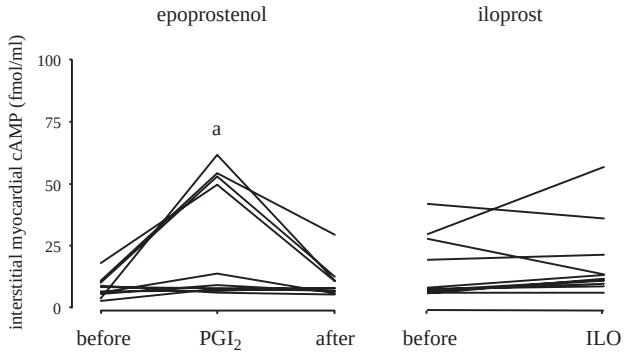


Fig. 5. Left ventricular end diastolic compliance was not changed significantly by the infusion of the vasodilators sodium nitroprusside, adenosine, epoprostenol and iloprost.



[a] p<0.05 vs. before, Wilcoxon signed rank test

Fig. 6. Epoprostenol raised the left myocardial interstitial cyclic adenylic acid (cAMP) significantly and reversibly (7.60 to 13.87 fmol/ml, $p<0.05$, $n=10$). Iloprost tended to increase left myocardial interstitial cAMP (7.56 to 11.66 fmol/ml, $n=11$). Single cases are presented.

The question whether the process of relaxation is load-dependent or not is discussed controversially (Brutsaert and Sys, 1989; Varma et al., 1989). In the present study diastolic aortic pressure was reduced to 75% with the four vasodilators. Yet, preload as measured by LV end diastolic volume was significantly reduced only during the infusion of epoprostenol. In contrast, adenosine and sodium nitroprusside reduced the maximum velocity of left ventricular pressure drop markedly. Therefore, adenosine and sodium nitroprusside worsened early active isovolumic relaxation, also directly compared to either iloprost or epoprostenol during equal reduction in diastolic aortic pressure.

Yet, these alterations in isovolumic relaxation did not affect the late passive phase of relaxation and filling as assessed by the end diastolic compliance in the present study in the intact left ventricle.

4.3. Clinical implications

The reduction of early active isovolumic relaxation, as caused by adenosine and sodium nitroprusside suggests that these drugs impair the diastolic function and may finally deteriorate global heart function. On the contrary, early active isovolumic relaxation remained stable during the infusion of the prostaglandin I_2 analogues epoprostenol and iloprost. As the current experimental study investigated the effects of vasodilators on left ventricular diastolic function of the *intact* myocardium in vivo, the impact of the current findings remains to be evaluated in the failing heart in vivo.

The infusion of adenosine and sodium nitroprusside impaired the early phase of left ventricular isovolumic relaxation. In contrast, the early active isovolumic relaxation properties of the left ventricle were preserved during the infusion of the prostaglandin I_2 analogues. Despite the reduced diastolic filling time as caused by vasodilator induced tachycardia during the infusion of sodium nitro-

prusside, epoprostenol, and iloprost, the passive phase of relaxation and filling of the intact left ventricle remained unchanged at the end of diastole in the intact myocardium.

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First part of the results of the present study focussing on *systolic left ventricular function* has been published recently.

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References

Baan, J., van der Velde, E.T., de Bruin, H.G., Smeenk, G.J., Koops, J., van Dijk, A.D., Temmerman, D., Senden, J., Buis, B., 1984. Continuous measurement of left ventricular volume in animals and humans by conductance catheter. Circulation 70, 812–823.

Boudoulas, H., Rittgers, S.E., Lewis, R.P., Leier, C.V., Weissler, A.M., 1979. Changes in diastolic time with various pharmacologic agents. Implication for myocardial perfusion. Circulation 60, 164–169.

Brutsaert, D.L., Sys, S.U., 1989. Relaxation and diastole of the heart. Physiol. Rev. 69, 1228–1315.

Gillebert, T.C., Sys, S.U., Brutsaert, D.L., 1989. Influence of loading patterns on peak length–tension relation and on relaxation in cardiac muscle. J. Am. Coll. Cardiol. 13, 483–490.

Kisch-Wedel, H., Kemming, G., Meisner, F., Flondor, M., Kuebler, W.M., Bruhn, S., Koehler, C., Zwissler, B., 2003. The prostaglandins epoprostenol and iloprost increase left ventricular contractility in vivo. Intensive Care Med. 29, 1574–1583.

Macov, F., Fagard, R., Mortelmans, L., Claeys, K., Amery, A., 1992. Effects of baroceptor and cardiopulmonary reflexes on neural control of heart rate in normal man, as assessed by power spectral analysis. Clin. Auton. Res. 2, 367–371.

Mirsky, I., 1984. Assessment of diastolic function: suggested methods and future considerations. Circulation 69, 836–841.

Monrad, E.S., McKay, R.G., Baim, D.S., Colucci, W.S., Fifer, M.A., Heller, G.V., Royal, H.D., Grossman, W., 1984. Improvement in indexes of diastolic performance in patients with congestive heart failure treated with milrinone. Circulation 70, 1030–1037.

Müller, M., Burgdorff, T., Jansen, B., Singer, E.A., Agneter, E., Dorner, G., Brunner, M., Eichler, H.G., 1997. In vivo drug-response measurements in target tissues by microdialysis. Clin. Pharmacol. Ther. 62, 165–170.

O'Brian, J.A., Strange, R.A., 1975. The release of adenosine 3′:5′-cyclic monophosphate from the isolated perfused rat heart. Biochem. J. 152, 429–432.

Parker, J.D., Aardal, S., Landzberg, J.S., Bittl, J.A., Mirsky, I., Collucci, W.S., 1991. Effects of β-adrenergic stimulation with dobutamine on isovolumic relaxation in the normal and failing human left ventricle. Circulation 84, 1040–1048.

Schertel, E.R., 1998. Assessment of left-ventricular function. Thorac. Cardiovasc. Surg. 46 (Suppl), 248–254.

Taylor, A.L., Ziesche, S., Yancy, C., Carson, P., D'Agostino Jr., R., Ferdinand, K., Taylor, M., Adams, K., Sabolinsky, M., Worcel, M., Cohn, J.N., 2004. African-American heart failure investigators. N. Engl. J. Med. 351, 2048–2057.

Varma, S.K., Owen, R.M., Smucker, M.L., Feldman, M.D., 1989. Is τ a preload independent measure of isovolumic relaxation? Circulation 80, 1757–1765.

Wallace, A., Lam, H.W., Mangano, D.T., 1995. Linearity, load dependence, hysteresis, and clinical associations of systolic and diastolic indices of left ventricular function in man. Multicenter Study of Perioperative Ischemia (McSPI) Research Group. J. Card. Surg. 10, 460–467.

Zwissler, B., 2000. Acute right heart failure. Etiology—pathophysiology—diagnosis—therapy. Anaesthesist 49, 788–808.