

Improved Ventricular Function during Inhalation of PGI₂ Aerosol Partly Relies on Enhanced Myocardial Contractility

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Key Words

Inhalation therapy · Prostacyclin · Aerosol · Pulmonary hypertension · Vasodilators · Myocardial contraction

Abstract

Inhaled prostacyclin (PGI₂) aerosol induces selective pulmonary vasodilation. Further, it improves right ventricular (RV) function, which may largely rely on pulmonary vasodilation, but also on enhanced myocardial contractility. We investigated the effects of the inhaled PGI₂ analogs epoprostenol (EPO) and iloprost (ILO) on RV function and myocardial contractility in 9 anesthetized pigs receiving aerosolized EPO (25 and 50 ng·kg⁻¹·min⁻¹) and, consecutively, ILO (60 ng·kg⁻¹·min⁻¹) for 20 min each. We measured pulmonary artery pressure (PAP), RV ejection fraction (RVEF) and RV end-diastolic-volume (RV-EDV), and left ventricular end-systolic pressure-volume-relation (end-systolic elastance, E_{es}). EPO and ILO reduced PAP, increased RVEF and reduced RVEDV. E_{es} was enhanced during all doses tested, which reached statistical significance during EPO_{25ng} and ILO, but not during EPO_{50ng}. PGI₂ aerosol enhances myocardial contractility in healthy pigs, contributing to improve RV function.

Introduction

The inhalation of short-acting vasodilator drugs is attractive to treat severe respiratory and cardiac disease: They may cause effective dilation of vessels within the lungs without affecting vascular tone in the systemic vasculature [1, 2]. Prostacyclin (PGI₂) is ubiquitous in the human body. Originally, it has been shown to safely, effectively and selectively dilate pulmonary vessels in experimental and clinical pulmonary hypertension of various origins [3–6]. Finally, PGI₂ and its analogs, epoprostenol (EPO) and the longer acting iloprost (ILO), have been shown to improve pulmonary hemodynamics, right heart function, and exercise capacity in patients with primary pulmonary hypertension [4, 7]. So far, investigators have attributed the positive effects of inhaled PGI₂-analogues mainly to the reduction in right ventricular afterload and – to some extent – of preload, as well.

However, a reduction of afterload alone may not sufficiently explain the pronounced improvement in stroke volume, observed after administration of PGI₂-analogues when compared to other pure vasodilators [8]. Since PGI₂ exerts its effects on pulmonary vascular tone via elevation of intracellular levels of cyclic adenosine monophosphate in the vascular smooth muscle cells, an additional cyclic

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adenosine monophosphate-mediated positive inotropic effect of PGI₂-analogs has been suggested [9, 10]. In a recent experimental investigation, we have demonstrated that the *intravenous* administration of both EPO and ILO equally increases left ventricular myocardial contractility qualifying PGI₂-analogs as inodilators [11]. This effect was accompanied by an increase of cyclic adenosine monophosphate in the interstitial dialysate obtained from the apex of the left ventricular wall [12].

It is, however, still unknown, if such a positive inotropic effect on the heart is also present after *inhalation* of PGI₂-analogs in the absence of pulmonary hypertension. Therefore, the aim of the present experimental study was to test the hypothesis that inhalation of aerosols of the PGI₂-analogs, EPO and ILO in clinically relevant concentrations (1) improves right ventricular function and (2) that this effect is caused by an improvement of myocardial contractility *in vivo*.

Materials and Methods

Study Design

The study was approved by the Bavarian Government. It was conducted in 9 pigs (German landrace, body weight 30 ± 5 kg), which were treated and housed in accordance with the 'Principles of Laboratory Animal Care' (NIH-publication No. 86-23, revised version 1985).

Anesthesia

After one night's starvation with free access to water, intramuscular premedication was performed with midazolam ($1 \text{ mg} \cdot \text{kg}^{-1}$) and ketamine ($10 \text{ mg} \cdot \text{kg}^{-1}$). Anesthesia was induced by intravenous (i.v.) injection of fentanyl ($20 \mu\text{g} \cdot \text{kg}^{-1}$) and propofol ($2 \text{ mg} \cdot \text{kg}^{-1}$), muscular paralysis by the use of vecuronium ($0.4 \text{ mg} \cdot \text{kg}^{-1}$ i.v.). All animals were placed in supine position and endotracheally intubated. Mechanical ventilation was performed on FiO₂ 0.5 to preserve normocapnia (Servo 900 B; Siemens, Solna, Sweden). Balanced anesthesia and muscular paralysis were maintained by the inhalation of isoflurane (2–3 vol %) and nitrous oxide (FiNO₂ 0.5), and by continuous i.v. infusion of fentanyl ($40 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ i.v.), as well as infusion of vecuronium ($0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ i.v.). Normothermia was ensured by means of a warming pad and a warming lamp. Insensible fluid losses were replaced by Ringer solution ($15 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ i.v.).

Surgical Preparation and Instrumentation

To minimize the surface of the artificial airway and enable effective inhalation of drug aerosols, we performed tracheostomy in all animals. For collection of arterial and mixed-venous blood samples and for determination of cardiac output using the thermal-dye method, a femoral arterial introducer sheath (8.5 F; Arrow, Reading, Pa., USA) and a fast response thermodilution PA catheter (7.5 F; REF-1™, Baxter, Irvine Calif., USA) were inserted. For arterial pressure recordings, an aortic tip-manometer catheter (PC 350; Millar Instruments, Tex., USA) was placed. For determination of

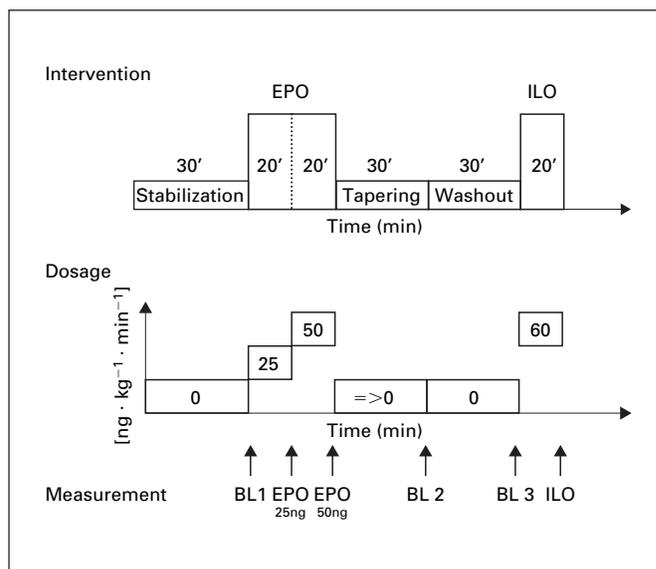


Fig. 1. Depiction of the interventions undertaken, the dosages administered and the measurements taken during the course of the protocol as delineated in the text. BL = Baseline.

myocardial contractility, a combined conductance/tip-manometer catheter (7 F, 12 Electrodes; CardioDynamics, Leiden, The Netherlands) and a Fogarty catheter (22 F; Baxter, Irvine, Calif., USA) were inserted into the left ventricle and the inferior vena cava, respectively. A central venous catheter (14 G; Arrow) via femoralis allowed for continuous drug administration. Catheter positions were verified by fluoroscopy.

Interventions and Measurements

The instrumentation was followed by a stabilization period of 30 min. A first set of baseline measurements was obtained thereafter. We then administered aerosolized EPO (25 and $50 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and ILO ($60 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in consecutive order, over a period of 20 min each. EPO and ILO were dissolved in normal saline; the total sodium chloride concentration of the resulting test solutions did not differ from isotonic saline. EPO was not abruptly withdrawn but slowly tapered down over 30 min. After the termination of EPO, we allowed for an additional washout period of 30 min (fig. 1).

Aerosol Production, Determination of Droplet Size and Aerosol Deposition

The PGI₂-analogs, EPO (Flolan™, Glaxo-Wellcome, Versailles, France) and ILO (Ilomedin™, Schering, Berlin, Germany) were administered with a standard jet nebulizer (Nebulizer 945™, Siemens, Erlangen, Germany) in combination with a nebulizer chamber (Micro Cirrus™, Intersurgical, Wokingham, Berkshire, UK). To quantify the amount of aerosol delivery, the exact turnover of a test solution in the Micro Cirrus™ chamber was determined with each single drug application and the concentration of the nebulizer content was calculated accordingly. In pilot studies, we determined the mass median aerodynamic diameter of the aerosol generated

by the Micro Cirrus™ chamber with a Stoeber centrifuge [13] and determined regional distribution of inhaled particles with a diameter of 1 μm using the method described by Hoegl et al. [14]. An aerosol of fluorescent microspheres (1 μm Fluospheres®; Molecular Probes Europe, Leiden, The Netherlands) was inhaled in an anesthetized pig. After sacrifice, the lung was removed and dissected (264 samples). Regional pulmonary deposition of microspheres was calculated from tissue sample fluorescence, which was measured with a luminescence spectrophotometer. Special software was allowed for 3D reconstruction of regional normalized aerosol deposition (CSD solutions; Oberpfaffenhofen, Germany) [15].

Measurements

A total of three baseline measurements (BL) were performed – prior to application of EPO, after tapering of EPO, and after wash-out prior to ILO. Further measurements were performed after 20-min inhalation of EPO (25 and 50 ng·kg⁻¹·min⁻¹) and ILO (60 ng·kg⁻¹·min⁻¹). There was no control measurement after termination of ILO due to its long half-life time (fig. 1).

Systemic Hemodynamics and Right Ventricular Function

Pulmonary arterial pressure was measured with a pulmonary artery catheter and a Statham pressure transducer. Left ventricular pressure was recorded online at 250 Hz (DasyLab; Datalog, Mönchengladbach, Germany) with the conductance/tip-manometer catheter. Cardiac output [16], right ventricular ejection fraction and end-diastolic volume were determined with fast response thermodilution (REF-1™ Ejection Fraction/-Cardiac Output Computer; Baxter, Santa Ana, Calif., USA). Central venous pressure (CVP) was measured via the proximal catheter lumen.

Myocardial Contractility

Myocardial contractility was estimated (1) from the first derivative of left ventricular pressure over time and (2) from pressure-volume loops of the left ventricle, which were registered during a preload reduction maneuver during shortly suspended ventilation (20 s). When a Fogarty catheter is rapidly inflated in the vena cava, left ventricular volume and pressure decline in a progressive manner. Simultaneously, left ventricular pressure and volume are registered online at 250 Hz (Meilhaus Electronic, Puchheim, Germany), allowing establishment of pressure-volume loops for consecutive single heart beats in the presence of progradient unloading. The end-systolic points at the end of left ventricular ejection period result in a straight line, the end-systolic pressure-volume relationship (ESPVR). The slope of ESPVR, the end-systolic elastance (E_{es}), is a relatively load independent measure of left ventricular contractility. ESPVR and E_{es} were obtained from calculated left ventricular volume derived from the conductance signal (see Appendix). At each baseline measurement 5 ml of hypertonic sodium chloride was injected to assess parallel conductance at a period of suspended ventilation. Estimation of parallel conductance is described in detail elsewhere. Parallel conductance was newly assigned during every baseline measurement. During preload reduction, the first three beats were excluded (change of parallel conductance by the filled right ventricle). In absence of ectopic beats, data were accepted. Linear regression analysis of the end-systolic points of all beats registered during preload reduction was performed with a special software (Conduct-PC, Version V720.1; CardioDynamics) and the slope of ESPVR, E_{es} was delivered.

Blood Samples and Calculations

Arterial and mixed-venous blood samples were taken simultaneously in air-free syringes, cooled and analyzed instantly in duplicate; pO₂ and pCO₂ were measured with a blood gas analyzer (Chiron Diagnostics, Fernwald, Germany). Hemoglobin concentration and hemoglobin O₂ saturation were measured by absorbance spectrophotometer (Instrumentation Laboratory, Lexington, Mass., USA). Pulmonary shunt fraction and dead space ventilation were calculated according to standard formula.

Statistics

Data are presented as median and semi-interquartile range MED (Q3-Q1/2). The three baseline measurements were tested for changes over time resulting from the crossover design (rANOVA). In each single set of data, the difference between control values and values which were obtained during inhalation of EPO and ILO, respectively, were calculated. The sum of these differences generated for one of the test substances was tested with a Signed-Rank test. The α-error threshold was set to 5%.

Results

Aerosol Delivery, Data Acquisition and Stability of the Model

The mean turnover of the jet nebulizer and the aerosol chamber was 0.2 ml·min⁻¹ of test solution at an O₂ flow rate of 8 liters·min⁻¹. The MMAD of the aerosol droplets denoted 1.1 μm with a geometric standard deviation of 1.6. Tentative nebulization of fluorescent microspheres (1 μm) resulted in homogenous regional distribution of fluorescence (Σ 2% deposition, distribution in fig. 2). In 9 animals, EPO and ILO were administered. Alterations of alveolar ventilation, pulmonary mechanics and gas exchange were absent (NS) (table 1). All parameters were unchanged during the three baseline measurements (NS).

Right Ventricular Function

We observed a significant reduction of pulmonary arterial pressure during inhalation of low-dose EPO (-13%, $p < 0.05$), high-dose EPO (-12%, $p < 0.05$), and ILO (-14%, $p < 0.05$). At the same time, right ventricular ejection fraction increased and right ventricular end-diastolic volume was reduced with high-dose EPO (+16%, $p = 0.05$ and -13%, $p < 0.05$) and ILO (+24%, $p < 0.05$ and -7%, $p < 0.05$), but not with low-dose EPO (+13% and -24%, NS). Right ventricular stroke volume and central venous pressure remained unchanged (table 1).

Myocardial Contractility

Both inhaled EPO and ILO enhanced myocardial contractility in presence of unchanged left ventricular and

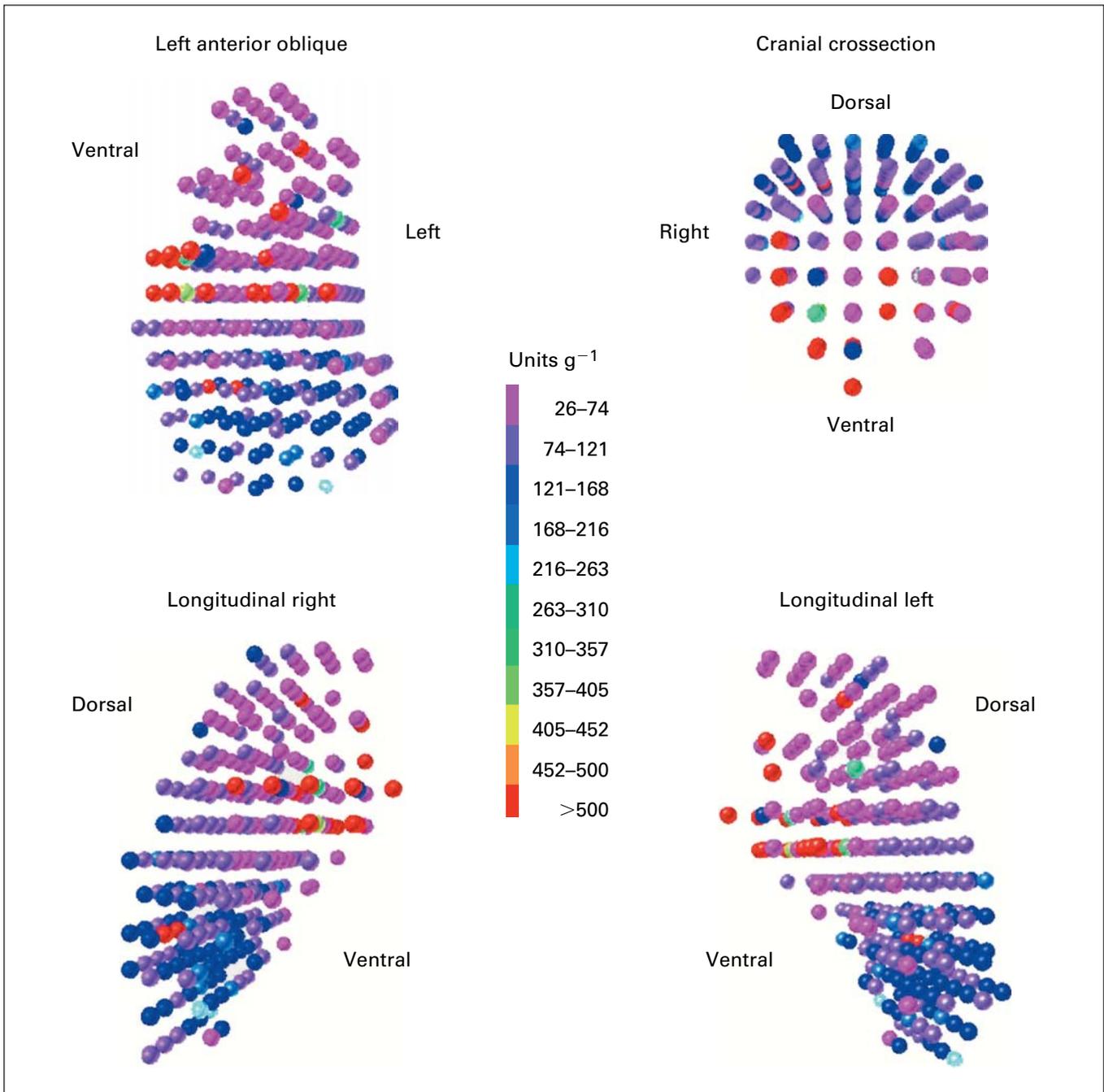


Fig. 2. A suspension of fluorescent microspheres was aerosolized and inhaled in a single anesthetized, tracheotomized and mechanically ventilated pig. The figure depicts regional deposition distribution of the inhaled microspheres (diameter of 1 μm), which was obtained ex vivo after sacrifice and postmortem removal of the whole lung (96 h of drying at CPAP 20 cm H_2O). The dried lung was dissected hierarchically (264 samples). Regional pulmonary deposition of microspheres was calculated from tissue sample fluorescence which was measured automatically with a luminescence spectrophotometer (Perkin Elmer). Deposition is expressed by specific fluorescence per tissue sample weight. Special software allowed

for three-dimensional reconstruction of the lung. The figure depicts the normalized regional 3D distribution of microspheres in the reconstructed lung in false tinting (CSD solutions; Oberpaffenhofen). The color scale illustrates allotment of colors to specific regional fluorescence intensity as expressed per sample weight (arbitrary units g^{-1}). Distribution of regional microsphere deposition was tested in a single pig under the described experimental conditions. All investigated tissue contained fluorescence. Increased fluorescence was observed in the dorsobasal regions of the lower lobes and in the direct neighborhood of the main bronchi.

Table 1. Cardiovascular and respiratory parameters

	BL 1	EPO ₂₅ ng	EPO ₅₀ ng	BL 3	ILO
HR, min ⁻¹	117 ± 15	114 ± 7	113 ± 4	123 ± 21	122 ± 18
CI, min ⁻¹ · m ⁻²	4.6 ± 0.8	4.2 ± 1.0	4.4 ± 0.8	5.6 ± 1.0	5.7 ± 0.7
MAP, mm Hg	111 ± 9	110 ± 9	109 ± 10	99 ± 12	101 ± 9
LVEDV, ml	38 ± 11	54 ± 13	47 ± 12	45 ± 16	44 ± 16
CPP, mm Hg	68 ± 9	70 ± 8	64 ± 12	57 ± 7	58 ± 7
PAP, mm Hg	28 ± 2	25 ± 3 [‡]	25 ± 3 [‡]	29 ± 5	24 ± 3 [‡]
RVEF, %	31 ± 4	35 ± 5	36 ± 3 ^{0.05}	34 ± 3	42 ± 4 [‡]
RVEDV, ml	98 ± 0	92 ± 3	68 ± 5 [‡]	113 ± 25	105 ± 20 [‡]
CVP, mm Hg	9 ± 2	11 ± 2	11 ± 1	12 ± 3	12 ± 2
RVSV, ml	37 ± 8	37 ± 3	39 ± 6	40 ± 8	44 ± 11
AWP _{peak} mm Hg	34 ± 3	34 ± 2	32 ± 2	32 ± 4	32 ± 5
TV, ml	289 ± 47	311 ± 32	311 ± 32	271 ± 32	260 ± 93
V _d /V _t , %	18 ± 5	12 ± 4	14 ± 4	18 ± 11	21 ± 3
QsQt, %	24 ± 3	25 ± 1	26 ± 3	26 ± 1	31 ± 3
PaCO ₂ , Torr	37 ± 1	37 ± 4	37 ± 4	37 ± 3	40 ± 3
PaO ₂ , Torr	199 ± 41	187 ± 35	161 ± 37	159 ± 26	154 ± 32

All values are given as median ± semi-interquartile range MED ± (Q3-Q1)/2.

HR = Heart rate; CI = cardiac index; MAP = mean arterial pressure; LVEDV = left ventricular end-diastolic volume; CPP = coronary perfusion pressure; PAP = pulmonary arterial pressure; RVEF = right ventricular ejection fraction; RVEDV = right ventricular end-diastolic volume; CVP = central venous pressure; RVSV = right ventricular stroke volume; AWP_{peak} = peak airway pressure; TV = tidal volume; V_d/V_t = dead space ventilation; QsQt = pulmonary shunt fraction; PaCO₂ = arterial carbon dioxide partial pressure; PaO₂ = arterial oxygen partial pressure.

[‡] p < 0.05 versus previous time point of measurement.

aortic pressure: E_{es} was enhanced with low-dose EPO (E_{es} +18%, p < 0.05), but not with high-dose EPO (E_{es} +13%; NS). The increase of E_{es} was pronounced upon the inhalation of ILO (+64%, p < 0.05). Dp/Dt_{max} increased with high-dose EPO (+4%, p 0.05) and with ILO (+6%, p < 0.05), whereas cardiac index improved only slightly after ILO (+2%, p < 0.05) (fig. 3, 4).

Discussion

Main Results

Recently, we described for the first time the positive inotropic effect of intravenous PGI₂ in an in vivo experimental model [11], thereby confirming previous in vitro data [9, 10, 17]. The main novel finding of the present study is that also *inhalation* of the aerosolized PGI₂-analogs EPO and ILO increases myocardial contractility. This is of particular significance, since inhalation of PGI₂-analogs is the clinically preferred mode of administration.

In the previous i.v. study several concerns had been raised, since i.v. EPO induced reflex tachycardia and hy-

potension. It could not be completely ruled out that the observed increase of E_{es} had been elicited by a humoral sympathoadrenergic response to hypotension rather than by an intrinsic positive inotropic effect of the prostanoids. In the present study, we observed no hypotension, no tachycardia or sympathomimetic reflex, definitely suggesting that the significant increase of E_{es} reflects a true increase in contractility upon inhaled EPO and ILO. In addition, the new data show that the positive inotropic effect is independent of the route of administration.

Mechanism of Action

There are two main mechanisms which could explain a positive inotropic effect of inhaled PGI₂-analogs. First, pharmacologically active PGI₂ might reach the left ventricular myocardium via 'spill over' to the systemic circulation [18] and might directly act on the cardiomyocyte. Systemic effects after spill over of inhaled PGI₂ have been previously reported [6, 19]. A direct action of EPO and ILO on the cardiomyocyte could result from activation of a PGI₂ receptor that interacts with adenylate cyclase. Both, EPO and ILO, are binding to myocardial prostaglandin receptors (EP₃) and do inhibit adenylate

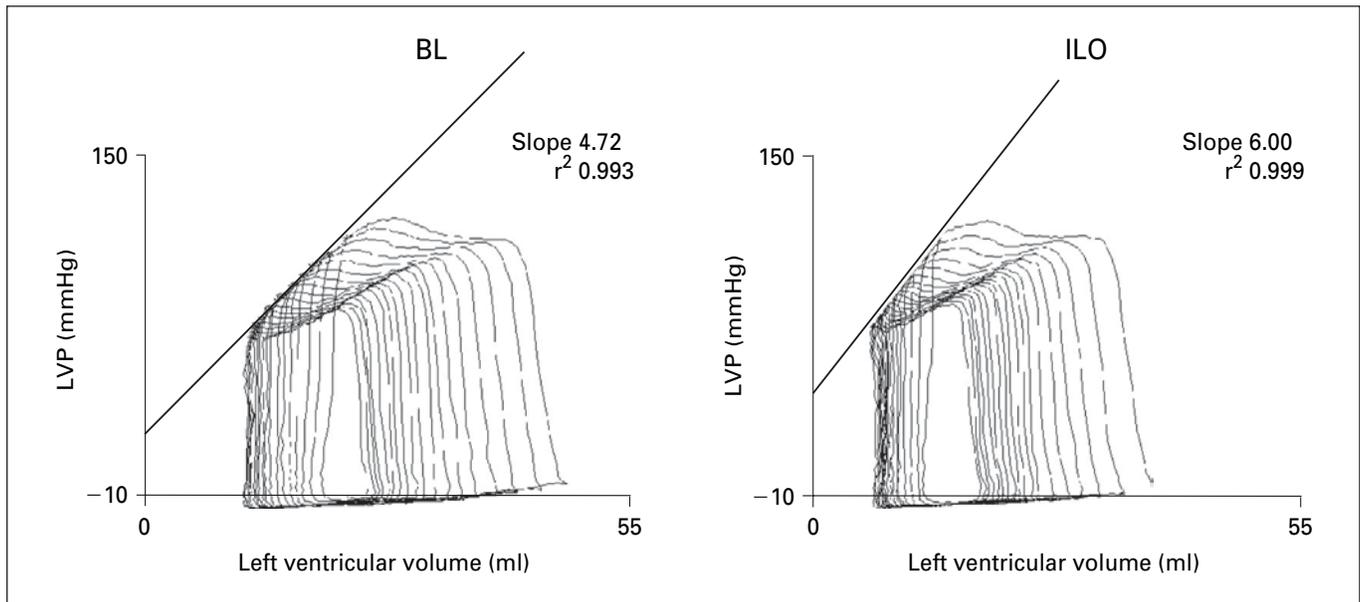


Fig. 3. Depiction of two original registrations of left ventricular pressure (LVP, y-axis) plotted against left ventricular volume (x-axis) during two preload reduction maneuvers: at baseline (BL, left panel) and during inhalation of iloprost-trometamol (ILO, 60 ng·kg⁻¹·min⁻¹, right panel). During each maneuver, a Fogarty balloon catheter is inflated in the inferior caval vein. The maneuver encompasses approximately 20 complete heart cycles. Every single pressure-volume-loop represents a single heart cycle with diastolic filling, systolic isovolumetric pressure increase, systolic ejection and diastolic relaxation of the left ventricle. During the maneuver, left

ventricular end-systolic pressure and volume are progressively reduced, the pressure-volume-loops shrink and shift to the left. If the end-systolic points of every single heart cycle are connected, they give a straight line. The straight line is defined as end-systolic pressure volume relation (ESPVR). The slope of this relation is defined as end-systolic elastance (E_{es}) and is a close estimation of load independent left ventricular contractility. In the depicted single experiment, end-systolic elastance increases notably from 4.72 to 6.00 ml·mm Hg⁻¹ during inhalation of iloprost-trometamol (ILO).

cyclase of the sarcolemma in the myocytes. Since experimental administration of β -blockers in isolated ventricles was shown to notably reduce the positive inotropic effects of PGI₂ [17], PGI₂ might also exert part of its inotropic action via β -receptors, which are also located on the surface of the myocytes.

Alternatively, the positive inotropic action of PGI₂ might result from a nerve stimulus generated locally in the lung in afferent autonomic sympathetic nervous fibers. The positive inotropic effect on the cardiomyocyte would finally be exerted via an efferent neuron as an autonomic nervous reflex [1]. Paradoxical tracheal constriction, as observed upon PGI₂ inhalation in dogs, is mediated by autonomic reflex activity via slow afferent neural structures (C fibers) in the terminal airways [20]. In analogy, the positive inotropic action might rely on such reflex activity. At present, the detailed mechanism remains to be elucidated.

Aerosol Delivery and Deposition

Since a jet nebulizer was used, respiratory volumes had to be adjusted to maintain airway pressures. However, our data show, that neither alveolar ventilation, nor oxygenation, nor pulmonary mechanics were affected by this fact. Aerosol production with our setup was in the range of what has previously been reported in experimental and clinical studies with jet and ultrasound nebulizers [6, 21]. Using the Stoeber centrifuge we give evidence that our aerosol droplets (MMAD 1.1 μ m) are likely to deposit in the alveolar region [22]. Moreover, as predicted by MMAD, homogenous regional distribution of particle deposition approximating a diameter of 1 μ m was confirmed by fluorescence measurements and reconstruction of the lung, as depicted in figure 2.

Measurement of Myocardial Contractility

Changes in the slope of the end-systolic pressure relationship (E_{es}) are load independent over a large range of

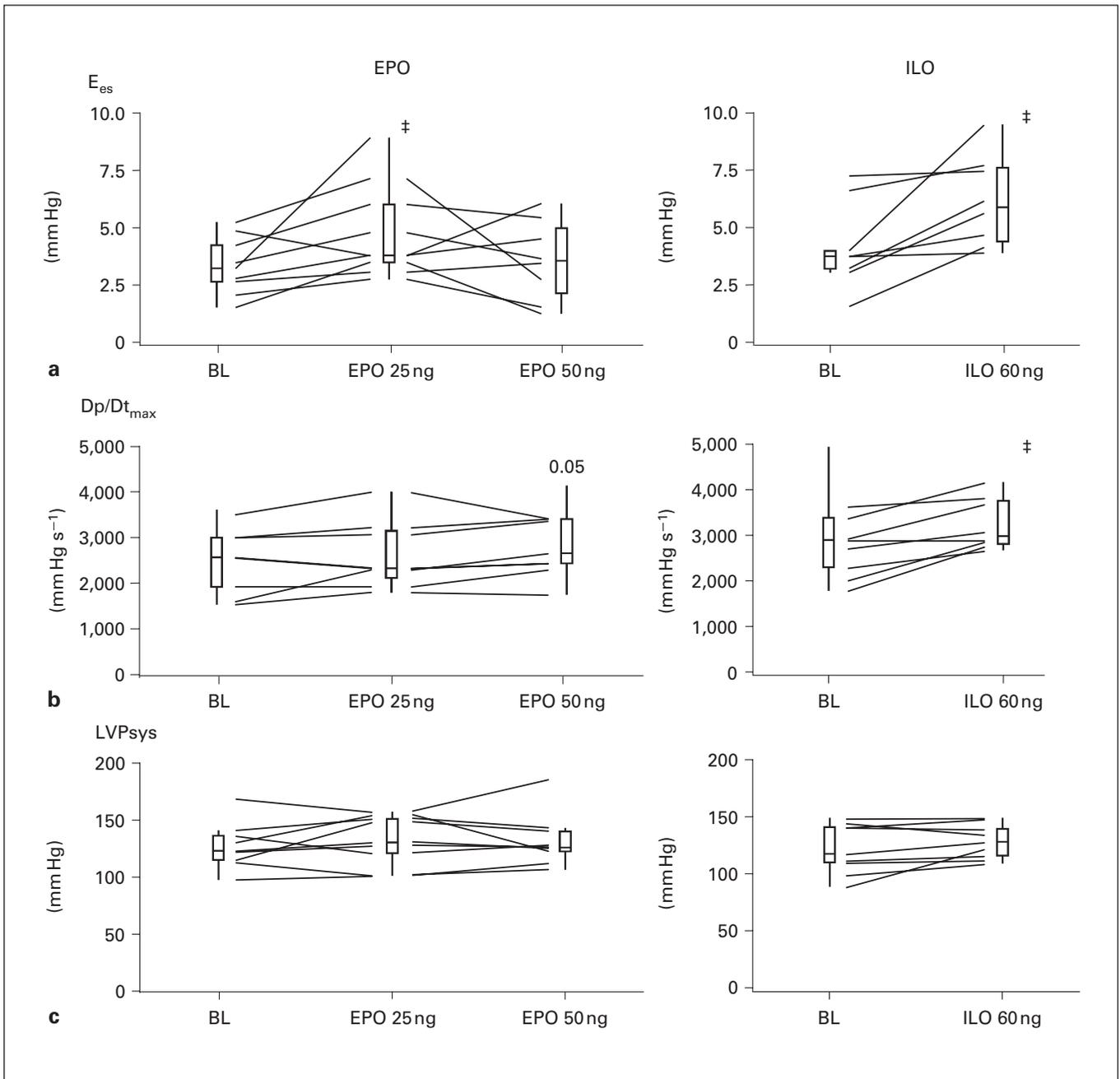


Fig. 4. Depiction of changes in end-systolic elastance (E_{es}) (a) and maximum of the first derivative of left ventricular pressure over time (Dp/Dt_{max}) (b) as well as left ventricular systolic pressure (c) as measures of myocardial contractility during inhalation of epoprostenol (EPO, 25 and 50 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and iloprost-trometamol (ILO, 60 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Each graphic gives data from all single experiments as well as box-and-whisker plots. The boxes depict

median, first and third interquartile, the whiskers 10th and 90th percentile. Changes as compared to baseline are indicated by a double dagger; the α -error threshold is set to 0.05; Wilcoxon Signed-Rank Test. The graphic depicts an improvement in all load-dependent and independent measures of ventricular performance and contractile myocardial force during inhalation of prostacyclin (PGI_2) analogs. ‡ $p < 0.05$ versus baseline (BL).

left ventricular pre- and afterload [23]. E_{es} thereby reflects the mechanical properties of the left ventricle in the state of complete contraction at the end of systole. To obtain valid information from E_{es} on the contractile state of the myocardium, ESPVR has to be linear. Yet, ESPVR may be nonlinear at extreme loading conditions [24]. Since ESPVR was nonlinear in the very beginning and at the end of preload reduction in our study, we excluded the first three beats and restricted data recording to the period of simultaneous constant pressure and volume decrease. This procedure resulted in very high data quality (mean r^2 of ESPVR 0.99 in 12 ± 6 beats), thereby ensuring linearity of ESPVR in our experiments.

Furthermore, tachycardia may hamper valid determination of E_{es} . Yet, changes in heart rate were absent in our study. Moreover, a reduction of aortic impedance has been shown to affect E_{es} [24]. Yet, neither mean arterial pressure nor arterial elastance as a measure of vascular load were changed throughout our experiments, suggesting that measured data of E_{es} are valid. Like others [25], we occasionally observed negative intercepts of ESPVR. This phenomenon may relate to the fact that absolute stroke volume was not referenced to a second method in our study. It may further relate to the loading range of the ventricle and to the calibration of the conductance signal. One point calibration of the parallel conductance by hypertonic saline is only an approximation, since parallel conductance may vary over time. However, the number of repeated saline injections has to be restricted in a single experiment to avoid increases in arterial sodium concentration. Therefore, parallel conductance was determined only with baseline measurements in our experiments. Changes in sodium concentrations were minimal, and interference of sodium concentration with contractility, therefore, is unlikely. Present baseline data corroborate previous work from our group in pigs and dogs [11, 26].

We describe an increase of load-independent contractility upon inhaled PGI₂-analogs. Of note, we observed a significant increase of E_{es} with low-dose EPO and with ILO, but not with high dose EPO. The reason for this finding is unclear. However, this does not question the proof of concept, since we observed positive inotropism after both inhaled EPO and ILO (including a nonsignificant trend towards increased E_{es} with high-dose EPO). We assume that the large scatter of our data explains this inconsistency.

Study Design

We investigated both inhaled EPO and ILO in the same experimental setup intending to reduce the number of lab-

oratory animals. Theoretically, the effects of the investigated drugs might overlap. However, half-life time of EPO is short (2–3 min). Baseline measurements were performed prior to EPO, after tapering of EPO and after washout prior to administration of ILO. All parameters investigated during BL 1, 2, and 3 were tested to exclude overlap effects (rANOVA; NS; data not shown). We did not randomize the interventions, since administration of ILO prior to EPO is not feasible due to the longer half-life time of ILO (elimination half-life time 20–30 min, half-life of effects 60 min). Intentionally, we investigated the effects of inhaled EPO and ILO on right ventricular function in the absence of pulmonary hypertension, because we aimed to characterize the effects of the PGI₂-analogs in the healthy heart. Clearly, further studies are required to re-evaluate the effects of inhaled prostanoids on contractility also under conditions of severe pulmonary hypertension.

Choice of Administered Dose

In the present study, changes in myocardial contractility were observed in a clinically relevant range of dosages of EPO and ILO. In the literature, EPO has been used in dosages ranging from 3 to 100 ng kg⁻¹ min⁻¹. ILO is administered at a dose of 5–20 µg, equaling 15–60 ng kg⁻¹ min⁻¹, leaving the nebulizer over a period of 20 min [1, 27]. Since a threshold dose at which to expect significant positive inotropism was not known, we did not investigate less than 25 ng kg⁻¹ min⁻¹ EPO or 20 µg ILO. Establishment of a dose-response relationship of the inotropic effect is nevertheless desirable.

Conclusion

Our data give evidence that inhaled EPO and ILO increase myocardial contractility in vivo, suggesting an inodilatory effect of PGI₂-analogs which is independent of the route of administration. An additional positive inotropic effect may contribute to the beneficial effects observed during short-term and long-term use of inhaled PGI₂-analogs in patients. In the future, inhaled PGI₂-analogs should be considered as inodilators rather than pure pulmonary vasodilators.

Acknowledgments

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Appendix

Equation 1: body surface area according to Holt et al. [16]

$$BSA = k \cdot BW^{2/3}, (k = 9)$$

BSA: body surface area; k: constant for landrace pigs; BW: body weight.

Equation 2: Left ventricular volume at time t, V(t)

$$V(t) = \rho \cdot L^2 \sum G_n(t) - \rho \cdot L^2 G^p$$

V(t): volume at time t; ρ : calibration factor for blood conductivity; $G_n(t)$: Conductance of one catheter segment at time t, where n = (1, ..., 4, 5) including only segments of the catheter which were placed in the left ventricle as indicated by segmental signals; G^p : parallel conductance of the surrounding tissue.

Equation 3: Coronary perfusion pressure

$$CPP \text{ (mm Hg)} = AOP \text{ diast} - LVEDP$$

CPP: coronary perfusion pressure; AOP diast: diastolic aortic pressure; LVEDP: left ventricular end-diastolic pressure.

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