Anesthesiology
81:1436–1444, 1994

Isoflurane Inhibits Hypoxic Pulmonary Vasoconstriction

An In Vivo *Fluorescence Microscopic Study in Rabbits*

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Background: **Contradictory results have been reported in previous studies investigating the effect of isoflurane on hypoxic pulmonary vasoconstriction by indirect approaches. The current study measured the effects of one-lung ventilation (1LV) and isoflurane 1.5% by direct visual observation of the pulmonary microcirculation.**

Methods: **Ten New Zealand White rabbits were anesthetized with intravenous thiopental, α-chloralose, and piritramid. Arterial, central venous, pulmonary arterial, left atrial, and airway pressures and cardiac output were recorded continuously. 1LV was facilitated by a bronchial blocker in the right main bronchus. A transparent window was implanted into the right thoracic wall for videofluorescence microscopy of the subpleural pulmonary microcirculation. After intravenous injection of fluorescein isothiocyanate–labeled red blood cells, vessel diameters, red blood cell flux, red blood cell velocity, and dynamic microhematocrit were measured in pulmonary arterioles and venules during two-lung ventilation and 1LV during baseline anesthesia and with supplementary isoflurane 1.5%.**

Results: **During intravenous anesthesia, 1LV caused significant reduction of vessel diameters and red cell flux and velocity and an increase in microvascular hematocrit in pulmonary arterioles and venules. The decreases in arteriolar diameters and red blood cell flux and velocity induced by 1LV were significantly attenuated by isoflurane as compared with those measured during baseline anesthesia ($P = 0.010$, $P = 0.029$ and $P = 0.047$). Accordingly, 1LV-induced reduction of venular red cell flux ($P = 0.023$) and velocity ($P = 0.036$) were less pronounced during isoflurane. Isoflurane caused a significant decrease in arterial pressure. Venous admixture increased and arterial oxygen tension decreased significantly during 1LV; the changes were more pronounced during 1LV with isoflurane 1.5% than during 1LV with baseline anesthesia.**

Conclusions: **1LV leads to a marked reduction of microvascular diameters and blood flow in the hypoxic lung. Isoflurane 1.5% inhibits hypoxic pulmonary vasoconstriction in pulmonary arterioles and increases regional blood flow in the hypoxic lung. (Key words: Anesthetics, volatile: isoflurane. Anesthetic techniques: one-lung ventilation. Lung(s): hypoxic pulmonary vasoconstriction. Measurement techniques: *in vivo* microscopy; microcirculation.)**

HYPOXIC pulmonary vasoconstriction (HPV) is an important factor influencing diversion of blood flow from atelectatic or hypoxic toward better aerated lung regions.[1] Thus HPV reduces venous admixture ($\dot{Q}_S/\dot{Q}_T$) and thereby minimizes arterial desaturation caused by regional pulmonary hypoxia. Because general anesthesia with mechanical ventilation and muscle paralysis induces a decrease in functional residual capacity,[2,3] thereby promoting atelectasis formation in dependent lung regions,[4,5] intact HPV is of considerable importance. This is especially true in anesthesia for thoracic procedures, where intentional unilateral atelectasis during one-lung ventilation (1LV) is used.[6,7]

HPV is significantly influenced by vasodilator drugs.[8–11] Volatile anesthetics, particularly isoflurane, are known to cause significant systemic vasodilation. As to the effects of volatile anesthetics on HPV, a considerable amount of experimental and clinical studies have yielded contradictory results. Inhibition of HPV by halothane, enflurane, and isoflurane was shown in isolated perfused lungs.[12–14] Sykes did not find a significant alteration of HPV with 0.5–1.5% halothane in dogs.[15] Early studies in dogs by Benumof and colleagues revealed a significant inhibition of HPV by isoflurane,[16] whereas the same group reported later on, that HPV is only slightly inhibited[17] by halothane and isoflurane in humans. In a study by Naeije *et al.* in dogs 0.5–1.5 minimum alveolar concentration isoflurane was shown to inhibit hypoxia-induced increases in pulmonary

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Received from the Institute of Anesthesiology and Institute for Surgical Research, Ludwig-Maximilians-University, Klinikum Grosshadern, Munich. Accepted for publication July 12, 1994. Supported by the Friedrich-Baur-Stiftung, Ludwig-Maximilians-University, Munich (to J.G.) and by BMFT 0706903 A5 from the Bundesministerium für Forschung und Technologie, Bonn (to A.E.G.). Presented in part at the annual meeting of the American Society of Anesthesiologists, San Francisco, California, October 1991.

vascular resistance (PVR), whereas halothane and enflurane had no effect.[18] From a clinical investigation Carlsson *et al.*[19] concluded that the effect of isoflurane on HPV is all but immeasurable at clinically relevant concentrations.

In all previous studies investigating the effects of volatile anesthetics on HPV the pulmonary vascular response to hypoxia was quantified using *indirect* parameters, such as pressure-flow-relationship,[12,18,20] intravascular pressure following vessel occlusion,[21] hypoxic-to-total lung perfusion ratio,[16,19,22] and $\dot{Q}_S/\dot{Q}_T$.[17,23,24] We have developed an experimental model allowing *direct* visual quantification of hypoxic vasoconstriction *in vivo* on the surface of the right lung of intact rabbits during 1LV as well as simultaneous macrohemodynamic measurements.[25] The current study was designed to quantify the changes in vessel diameters and microhemodynamic parameters in the hypoxic lung induced by 1LV during intravenous baseline anesthesia and by supplementary administration of 1.5% isoflurane during two-lung ventilation (2LV) and 1LV.

Materials and Methods

The study was carried out in 10 White New Zealand rabbits weighing 2,350–4,050 (mean 2,983) g after approval by the institutional animal research review board. The experimental procedure has been described elsewhere in detail.[25] In brief, after intravenous induction of anesthesia with thiopental (10–20 mg/kg body weight), tracheotomy and start of controlled mechanical ventilation (Sechrist Infant-Ventilator, Kontron, Eching, Germany; peak inspiratory pressure 12 cmH_2O, positive end-expiratory pressure 2 cmH_2O, mean airway pressure [AWP] 7 cmH_2O), baseline anesthesia was established by intravenous infusion of α-chloralose (50 mg/kg over a 30-min period) and injection of piritramid (1.5 mg/kg Dipidolor, Janssen GmbH, Neuss, Germany). No additional anesthetics were required throughout the experiments as indicated by continuous hemodynamic monitoring. Muscle relaxation was obtained by injection of 1 mg pancuronium (Pancuronium, Organon Technika, Freiburg, Germany) followed by continuous infusion of 0.6 mg/h. Catheters were advanced for continuous measurement of arterial, central venous, pulmonary arterial (PAP), and left atrial (LAP) pressures. Cardiac output was measured by means of an electromagnetic flow probe placed around the pulmonary artery. For implantation of a transparent window into the right thoracic wall the fourth and fifth ribs were partially resected over 3 cm. The transparent Teflon membrane of the window was superfused with warmed (37°C) Tyrode buffer solution, which was equilibrated with a normoxic gas mixture during 2LV and with a hypoxic gas mixture during 1LV to oxygen tensions of 80–100 and 30–40 mmHg, respectively.

For 1LV a 4F bronchial blocker (A1-07121, Arrow International, Reading, PA) was advanced through the ventilation tube into the right main bronchus with the blocker cuff placed precisely distal to the carina. The catheter lumen was flushed with saline and connected to a pressure transducer for monitoring of AWP. During 1LV mean AWP of the right lung was maintained at the same level as during 2LV (7 cmH_2O). For this purpose a thin Teflon catheter was advanced with its tip distal to the blocker cuff to inflate nitrogen into the blocked lung. The correct position of the bronchial blocker was verified by fiberoptic bronchoscopy (BF-N20, Olympus, Tokyo, Japan) during each experimental phase. Fiberoptic verification of the correct location of the inflation catheter was restricted to 2LV phases. However, free nitrogen gas flow was indicated during 1LV by constant AWP and persistent inflation of the hypoxic right lung. Ventilation of the left lung was continued throughout the experiments maintaining mean AWP at 7 cmH_2O.

For videomicroscopic investigation of the pulmonary microcirculation the animals were placed in the left lateral decubitus position on a movable table with a two-directional stepping motor (step width 25 μm; IXE.C, Phytron, Groebenzell, Germany). Morphometric and microhemodynamic measurements of subpleural pulmonary arterioles and venules were performed using a recently developed videofluorescence microscopic technique.[26] One percent fluorescein isothiocyanate (FITC) dextran aqueous solution (0.1 ml/kg) and 1 ml FITC-labeled red blood cells (RBC) were injected into the left atrial catheter to visualize vessel walls and blood flow. After FITC-labeled RBC injection a period of 30 min elapsed before microscopic studies were begun.

The microscopic images were recorded with a silicone-intensified video camera (C2400-08, Hamamatsu, Herrsching, Germany), stored by a U-matic video recorder (VO-5850; Sony, Munich, Germany) and analyzed off-line frame-by-frame using a digital image analysis system (Optimas, BioScan, Edmonds, WA). Repeated measurements of inner vessel diameters yielded an error of ± 1 μm. The velocity of labeled RBC was measured as the distance in axial direction of the vessel

the RBC moved per unit of time. RBC flux was calculated as the number of FITC-labeled RBC passing a predefined cross section of the vessel plane per unit of time multiplied by the labeled cell fraction as determined by flow cytometry (FACS analyzer; Becton-Dickinson, Heidelberg, Germany). Because the precision of microhemodynamic parameters depends on the number of cells included into measurement, analysis of FITC-labeled RBC was continued until the coefficient of variation was <5% for RBC flux and RBC velocity.

Macrohemodynamic measurements and videomicroscopy were performed during four experimental conditions, established in random order: 2LV and 1LV during baseline anesthesia and during additional administration of 1.5% isoflurane (Forene, Abbott, Wiesbaden, Germany). Micro- and macrocirculatory measurements were started 30 and 20 min after changing the anesthetic (baseline or isoflurane) and ventilatory (2LV or 1LV) regimen, respectively.

Statistical comparison of the changes induced by 1LV during administration of 1.5% isoflurane and during intravenous baseline anesthesia was performed by use of the *t* test. Additional exploratory analysis of differences between 2LV and 1LV as well as between baseline anesthesia and isoflurane was performed using one-way analysis of variance followed by *t* tests with correction of the α-level according to Newman-Keuls. Values are given as means ± standard error, and the level of significance was $P < 0.05$ unless otherwise indicated.

Results

Macrohemodynamics and Pulmonary Gas Exchange

Macrohemodynamic measurements and parameters of gas exchange are shown in table 1. Baseline hemodynamic data of the individual animals recorded after surgical preparation and a 30 min stabilization period did not differ significantly from those measured during 2LV during baseline anesthesia. Statistical analysis revealed no differences in heart rate, central venous pressure, and left atrial pressure between the experimental conditions. Administration of isoflurane caused a significant reduction of systemic arterial pressure both during 2LV and 1LV. PVR and $\dot{Q}_S/\dot{Q}_T$ increased during unilateral hypoxia as compared with 2LV whether or not isoflurane was administered. Corresponding to the increase in $\dot{Q}_S/\dot{Q}_T$, 1LV induced a significant reduction of arterial oxygen tension (Pa_{O_2}). During 1LV with isoflurane $\dot{Q}_S/\dot{Q}_T$ was significantly greater and Pa_{O_2} was significantly less than during 1LV during baseline anesthesia. Because inspiratory AWP was kept constant throughout the experiment, moderate respiratory acidosis developed during 1LV. However, the changes in arterial carbon dioxide tension did not reach statistical significance.

Microhemodynamic Parameters

Arterioles. Baseline diameters of pulmonary arterioles investigated in this study ranged from 21 to 51 μm. Unilateral hypoxia during intravenous anesthesia induced significant decreases in arteriolar diameter, RBC flux, and RBC velocity, by 11.1 ± 2.6%, 50.8 ± 9.2%, and 53.0 ± 8.9%, respectively, and an increase in microvascular hematocrit by 7.1 ± 21.9% (fig. 1). In two animals superficial arteriolar blood flow was completely absent during 1LV during baseline anesthesia but rapidly recovered after 2LV was resumed. In contrast, no absence of flow was observed during 1LV when isoflurane was administered, and arteriolar diameters were not significantly smaller during 1LV with isoflurane 1.5% as compared with 2LV with isoflurane 1.5%. The decreases in arteriolar diameters and RBC flux and velocity induced by 1LV were significantly inhibited by isoflurane as compared with intravenous baseline anesthesia (fig. 2, $P = 0.010$, $P = 0.029$, and $P = 0.047$).

Venules. Venular diameters, RBC flux, and RBC velocity decreased during 1LV during both anesthetic conditions compared with the corresponding 2LV phases (fig. 3). The changes in RBC flux and velocity were more pronounced during intravenous anesthesia than during supplementary isoflurane administration (fig. 2, RBC flux: $P = 0.023$, RBC velocity: $P = 0.036$).

Discussion

This is the first study providing direct measurements of the effects of the volatile anesthetic isoflurane on the hypoxic vascular response and the microhemodynamics in pulmonary arterioles and venules. Using *in vivo* microscopy in an intact animal model, we have shown that unilateral hypoxia induced by 1LV results in a decrease of arteriolar and venular diameters and in a reduction of RBC flux and velocity. Isoflurane 1.5% significantly inhibited the vascular and microhemodynamic effects of hypoxia.

Intravital Microscopy

Intravital microscopy is the only technique enabling simultaneous quantification of microvascular diameters

Table 1. Hemodynamic and Gas Exchange Parameters Measured during the Experimental Phases

Parameter	Baseline	$2LV_B$	$2LV_I$	$1LV_B$	$1LV_I$
AP (mmHg)	78 ± 4	83 ± 4	64 ± 4*	75 ± 3*	64 ± 4†
PAP (mmHg)	16 ± 0.7	17 ± 1.3	16 ± 1.0	18 ± 1.3*	19 ± 1.4‡
CVP (mmHg)	5.8 ± 0.7	5.5 ± 0.6	5.1 ± 0.5	6.0 ± 0.8	6.2 ± 0.9
LAP (mmHg)	5.0 ± 0.5	4.5 ± 0.8	4.6 ± 0.7	4.5 ± 0.9	4.5 ± 0.8
AWP LL (cmH_2O)	12/7/2	12/7/2	12/7/2	12/7/2	12/7/2
AWP RL (cmH_2O)	12/7/2	12/7/2	12/7/2	7	7
CO ($ml \cdot min^{-1}$)	187 ± 12	186 ± 14	201 ± 13	156 ± 17	166 ± 19‡
HR (min^{-1})	251 ± 8	258 ± 9	257 ± 6	249 ± 9	258 ± 12
SVR (mmHg · min/L)	360 ± 45	442 ± 40	301 ± 52*	488 ± 57	383 ± 52
PVR (mmHg · min/L)	57 ± 7	67 ± 5	57 ± 7	95 ± 10*	90 ± 12‡
*p*H	—	7.34 ± 0.01	7.32 ± 0.02	7.27 ± 0.03*	7.23 ± 0.03‡
Pa_{CO_2} (mmHg)	—	39.0 ± 2.4	41 ± 3.3	47.0 ± 3.4	49 ± 4.2
Pa_{O_2} (mmHg)	—	463 ± 14	456 ± 22	221 ± 22*	167 ± 23†‡
$\dot{Q}_S/\dot{Q}_T$ (%)	—	8.8 ± 1.1	10.5 ± 1.0	16.0 ± 1.3*	24.3 ± 2.4†‡

AP = arterial pressure; PAP = pulmonary arterial pressure; CVP = central venous pressure; LAP = left atrial pressure; AWP = airway pressure; LL = left lung; RL = right lung; CO = cardiac output; HR = heart rate; SVR = systemic vascular resistance; PVR = pulmonary vascular resistance; Pa_{CO_2} = arterial partial pressure of carbon dioxide; Pa_{O_2} = arterial partial pressure of oxygen; $\dot{Q}_S/\dot{Q}_T$ = venous admixture; $2LV_B$ = 2LV during intravenous baseline anesthesia; $2LV_I$ = 2LV during additional isoflurane administration; $1LV_B$ = 1LV during intravenous baseline anesthesia; $1LV_I$ = 1LV during additional isoflurane administration. Values are mean ± SEM.

* $P < 0.05$ *versus* $2LV_B$.

† $P < 0.05$ *versus* $1LV_B$.

‡ $P < 0.05$ *versus* $2LV_I$.

and blood flow in the pulmonary microcirculation *in vivo*. It is a direct approach for investigating the response of the pulmonary microvasculature to global or regional hypoxia and for measuring the effect of pathophysiologic conditions and of drugs on HPV.[25,26]

However, quantification of microcirculatory parameters by *in vivo* microscopy is restricted to vessels on the lung surface. Contradictory results have been reported as to whether superficial vessels are representative for the microcirculation lying deeper in the lung.

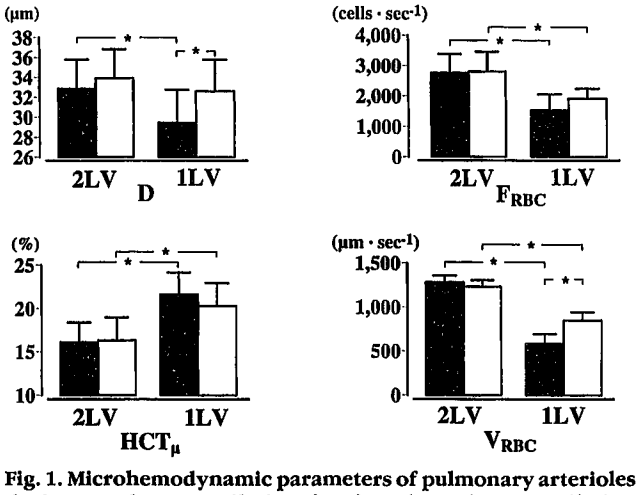

Fig. 1. Microhemodynamic parameters of pulmonary arterioles during two-lung ventilation (2LV) and one-lung ventilation (1LV) during baseline anesthesia (filled columns) and during isoflurane administration (open columns) (mean ± SEM). D = diameter; HCT_μ = dynamic microhematocrit; F_{RBC} = red blood cell flux; V_{RBC} = red blood cell velocity. *$P < 0.05$.

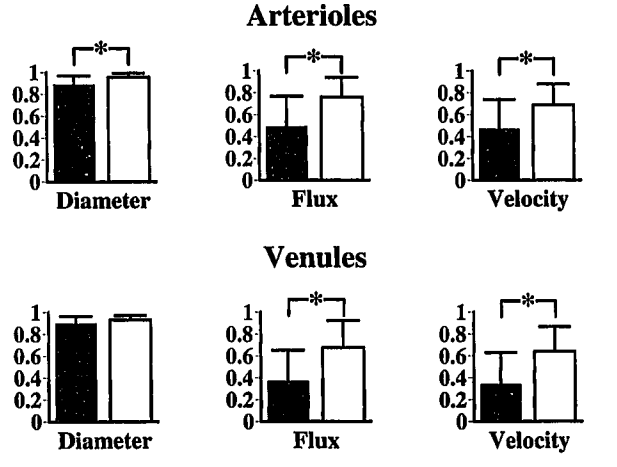

Fig. 2. One-lung/two-lung ventilation ratio of vascular diameters, red blood cell (RBC) flux, and RBC velocity in pulmonary arterioles and venules during baseline anesthesia (filled columns) and during isoflurane administration (open columns) (mean ± SEM). *$P < 0.05$.

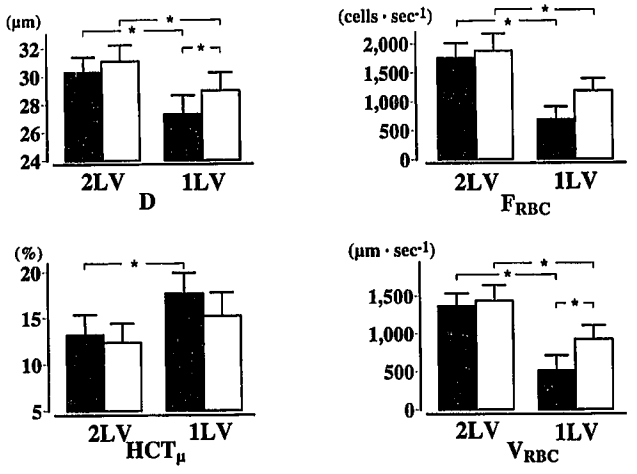

Fig. 3. Microhemodynamic parameters of pulmonary venules during two-lung ventilation and one-lung ventilation during baseline anesthesia (filled columns) and during isoflurane administration (open columns) (mean ± SEM). D = diameter; HCT_{μ} = dynamic microhematocrit; F_{RBC} = red blood cell flux; V_{RBC} = red blood cell velocity. *$P < 0.05$.

Subpleural capillary networks have been reported to be less dense than more interior networks.[27] However, inconsistent results were published as to a potential central-peripheral gradient of regional perfusion,[28–30] which would be expected, if these morphologic differences had functional significance. In a recent study on regional pulmonary blood flow during bilateral ventilation and unilateral hypoxia in lateral decubitus position,[31] we found identical changes in regional perfusion throughout the hypoxic (independent) lung, even in the uppermost slice corresponding to the area of microscopy in the current study. In accordance with others,[32] we therefore assume microhemodynamic measurements in subpleural vessels to reflect changes occurring in regions inside the lung.

Anesthetic Regimen

In our model a window is implanted into the thoracic wall to allow intravital microscopic investigation of the pulmonary circulation. Thus general anesthesia is an indispensable prerequisite. On the other hand, the anesthetic regimen may influence the results of hemodynamic studies. The synthetic opioid piritramid was combined with α-chloralose for intravenous baseline anesthesia to provide anesthesia and analgesia while avoiding side effects on the pulmonary circulation. Several studies *in vitro* and *in vivo* investigating the effect of opioids on HPV did not reveal an inhibitory potency.[13,33–36] α-Chloralose has been shown not to influence autonomic reflexes in animals[37,38] and is commonly considered an ideal anesthetic for cardiovascular studies.[39] Thus α-chloralose has recently been recommended when prolonged anesthesia is required for experimental studies of cardiovascular physiology.[40] According to hemodynamic data no additional anesthetics were required throughout the experiments. α-Chloralose produces several hours of anesthesia in cats when administered in the dosage used in our study.[41] Lambs have been shown not to recover from monoanesthesia induced by 30 mg/kg α-chloralose earlier than 4–6 h after administration.[42] α-Chloralose anesthesia is characterized by effective analgesia, whereas perception of auditory and tactile stimuli are less deeply depressed.[41] If an inadequate level of anesthesia had been indicated by hemodynamic parameters (increase of arterial pressure or heart rate) an additional dose of α-chloralose and piritramid would have been administered.

Statistical Evaluation

The current study was designed to investigate two hypotheses. First, we aimed at showing that isoflurane modifies the effect of 1LV. This was done by comparing the changes induced by 1LV in the presence of isoflurane with those found during baseline anesthesia using the paired *t* test. However, arterial oxygenation during 1LV is mainly determined by the fraction of cardiac output perfusing the hypoxic lung. Substances which induce vasodilation and increased blood flow in the nondependent lung during both 2LV and 1LV, may therefore unfavorably affect arterial oxygenation even if they do not significantly alter the relative changes induced by 1LV. Therefore the effects of 1LV and isoflurane were analyzed separately in addition by direct comparison between phases by means of one-way analysis of variance, followed by paired *t* test comparisons with correction of the α-level according to Newman-Keuls.

Macrohemodynamics

Macrohemodynamic baseline conditions are comparable to those reported recently using the same animal model.[26] Our results confirm the known vasodilatory effect of isoflurane in the systemic circulation. Hemodynamic changes in the pulmonary circulation during 1LV in the current animal study in rabbits, however, differ to those found in other animals and humans: Elliott *et al.*[43] reported an increase in PAP with decreasing

unilateral inspiratory oxygen fraction in dogs, ponies, and swine while cardiac output remained unchanged. Similar results were obtained earlier by Benumof and Wahrenbrock in dogs[16] and in humans.[17] Cardiac output and PAP did not change during 1LV in a clinical study by Rogers and Benumof,[24] whereas Carlsson *et al.*[19] found a significant increase in PAP induced by unilateral hypoxic ventilation both during intravenous and isoflurane anesthesia. In our study the increase in PVR due to unilateral HPV was not accompanied by a comparable increase in PAP, but by a decrease in cardiac output. Reduced venous return due to variable intrathoracic pressure may be excluded as a possible explanation for reduced cardiac output, because mean AWP in both lungs was identical during 1LV and 2LV. The results therefore suggest a reduced ability of rabbits to compensate for increased right ventricular afterload compared with other species.

Pulmonary Gas Exchange

HPV only partially reduces blood flow to hypoxic or atelectatic regions of the lung. Remaining shunt perfusion during 1LV increases $\dot{Q}_S/\dot{Q}_T$ and thus decreases Pa_{O_2}. The differences between changes of $\dot{Q}_S/\dot{Q}_T$ and Pa_{O_2} induced by 1LV during intravenous anesthesia and during isoflurane administration just missed the level of statistical significance ($\dot{Q}_S/\dot{Q}_T$: $P = 0.073$, Pa_{O_2}: $P = 0.102$). However, analysis of variance followed by multiple comparisons yielded a further increase of $\dot{Q}_S/\dot{Q}_T$ as well as a decrease of Pa_{O_2} when isoflurane was administered during 1LV. These results suggest greater shunt perfusion of the hypoxic lung during isoflurane administration, although increased blood flow to hypoxic segments of the dependent, ventilated lung may also have contributed to increased $\dot{Q}_S/\dot{Q}_T$. Because left-lung AWP was kept constant, an increase of arterial carbon dioxide tension was observed during 1LV. Conflicting results have been published concerning the effect of pH and carbon dioxide tension on the pulmonary vascular response to hypoxia. However, most authors concluded that respiratory acidosis does not significantly affect HPV,[44-46] because the enhancing effect of low pH on HPV is counteracted by CO_2-induced vasodilation.

One-lung Ventilation

During 1LV, the right main bronchus was occluded by a bronchial blocker and the lung was inflated with pure nitrogen to allow microscopy. Mean AWP of the right lung was kept at the same level as during 2LV (7 cmH_2O) to minimize mechanical effects on the pulmonary circulation. However, the forces acting on pulmonary microvessels during static inflation may differ from those during phasic inflation and deflation induced by mechanical ventilation. During positive pressure ventilation phasic changes of AWP are transmitted to the alveolar vessels. During inspiration alveolar capillaries are compressed thereby increasing arteriolar outflow resistance and augmenting driving pressure in the venous part of the pulmonary circulation. In contrast extraalveolar vascular resistance decreases during lung inflation, because these vessels are distended by outward traction of the surrounding tissue.[47] Opposite changes occur during expiration. Although the effects of unilateral static inflation *versus* bilateral ventilation on blood flow have not been investigated in detail so far, these factors should be taken into account for interpretation of regional perfusion.

Microhemodynamics

Previous studies investigating the effect of isoflurane on HPV and pulmonary gas exchange during unilateral hypoxia yielded conflicting results. These discrepancies may be attributed to the varying experimental approaches[7] using indirect parameters for assessment of the pulmonary vascular response to alveolar hypoxia. The purpose of the current study was therefore to evaluate the effect of isoflurane on HPV by direct measurements of vascular diameters and RBC kinetics in the pulmonary microcirculation of intact animals.

We have shown that arteriolar as well as venular diameters decrease in response to unilateral alveolar hypoxia during intravenous baseline anesthesia and that this response is significantly inhibited by 1.5% isoflurane in arterioles. Microhemodynamic parameters indicating local pulmonary blood flow (*i.e.*, RBC flux and velocity) decreased during unilateral hypoxia in arterioles and in venules both during intravenous and isoflurane anesthesia. Arteriolar and venular diameters decreased by approximately 10% during 1LV during intravenous baseline anesthesia. With isoflurane, however, only venules showed a significant decrease of vessel diameters, whereas arterioles constricted only slightly. Thus the decrease of vessel diameters in arterioles was significantly smaller during isoflurane than during baseline anesthesia, whereas the diameter ratio 1LV/2LV was not significantly different in venules. The most reasonable explanation for this finding is, that the inhibition of HPV by isoflurane is more prominent in arterioles than in venules. However, it cannot be ex-

cluded, that the diameter reduction in venules during 1LV is a passive phenomenon induced by decreased blood flow. Because a reduction of venular blood flow was present during baseline anesthesia and isoflurane, a passive mechanism of diameter reduction in venules would be in agreement with our data.

The current study highlights the importance of small pulmonary arterioles for an efficient HPV. In contrast to earlier deductions from indirect parameters, this is the first study which directly demonstrates this vascular reaction in mammalian pulmonary microvessels < 150 μm. However, active constriction in response to hypoxia has been proposed for all segments of the pulmonary microvasculature: Based on measurements of microvascular resistance, venous constriction has been proposed by Furnival *et al.*[48] and Morgan *et al.*[49] in dogs and more recently by Raj and Chen[50] in isolated lamb lungs. Morphometric *ex vivo* studies of Kapanci *et al.*[51] even indicated reduction of capillary diameters due to contractile interstitial cells. The majority of investigations, however, point to small pulmonary arteries as the main sites of HPV.[6,46,52-55] Using high-resolution CT Herold *et al.*[56] observed both constriction and dilation of arteries and veins > 300 μm in response to hypoxia. Furthermore recent angiographic studies by Al-Tinawi *et al.*[57] showed dilation of arteries > 800 μm during alveolar hypoxia at high pulmonary artery pressures suggesting that the main increase in vascular resistance occurs in more distal vascular segments. These data together with hemodynamic model calculations led them to postulate, that 'small' pulmonary vessels < 150 μm mainly account for the increase in pulmonary vascular resistance during alveolar hypoxia. Intravital microscopy provides much greater spatial resolution than CT and angiography and is the only method allowing assessment of these previous hypotheses by direct measurement of the hypoxia response of arterioles and venules in these 'vessels of interest' below 150 μm in diameter.

Arterioles and venules with baseline diameters ranging from 21–51 μm were included in the study, because reliable identification and differentiation of single fluorescently labeled RBC for measurement of velocity and flux is possible in this size range of microvessels only.[26] Criticism may arise concerning the active constriction of these small arterioles in response to hypoxia. To our knowledge, the distribution of vascular smooth muscles in pulmonary arteries of the rabbit has only been assessed by one study revealing muscular components in vessels down to 40 μm in diameter.[58] However, we found consistent decreases in vessel diameters with a pulmonary artery pressure comparable to the "high" pressure range of Al-Tinawi's study[57] supporting the conclusion that these small vessels significantly contribute to the increase of vascular resistance in hypoxic lung regions. This concept is further supported by previous intravital microscopic results obtained in the same experimental model: during global alveolar hypoxia induced by hypoxic ventilation (inspiratory oxygen fraction 0.12) a reduction of vessel diameters has been shown in pulmonary arterioles and venules of the same size range as in the current study, while RBC flux and RBC velocity simultaneously increased.[59] Because reduction of vessel diameters causes an increase in vascular resistance, higher RBC flux and velocity in these conditions indicate an increase in microvascular driving pressure. These results suggest that pulmonary arterioles and venules with baseline diameters down to less than 20 μm are able to actively constrict during alveolar hypoxia. However, these data do not exclude a contribution of pulmonary arterioles larger than those investigated in our study to the increase in pulmonary vascular resistance during hypoxia.

1LV caused a decrease of cardiac output. Lower cardiac output has to be considered as a possible contributory factor of reduced RBC flux and velocity during 1LV. However, the relative decrease of both microhemodynamic parameters by far exceeded the percent change in cardiac output, emphasizing the predominant role of regional vasoconstriction in the hypoxic lung. In addition we have previously shown that unilateral hypoxia results in a significant uniform redistribution of pulmonary blood flow toward the normoxic lung.[31]

In contrast to all previous techniques used to assess the influence of volatile anesthetics on HPV, intravital fluorescence microscopy allows simultaneous quantification of vessel diameters and of microhemodynamic parameters indicating the hemodynamic consequences of HPV on regional blood flow in the pulmonary microcirculation. During intravenous baseline anesthesia RBC flux and velocity decreased by 51% and 53% in arterioles and a comparable decrease was observed in venules. Interestingly, the extent of this decrease in microvascular blood flow during baseline anesthesia is in excellent agreement with previous macrohemodynamic findings.[23,60] Thus regional shunt perfusion is approximately 50% of baseline blood flow, because all of the remaining blood flow to the hypoxic lung is shunt flow. With isoflurane, RBC flux and velocity de-

creased by 24% and 31% in arterioles. This demonstrates that HPV is not completely inhibited by isoflurane. However, in arterioles as well as in venules, the decrease of RBC flux and velocity was significantly smaller than during baseline anesthesia. This finding directly proves an inhibition of HPV by isoflurane.

In agreement with previous results the dynamic microhematocrit in superficial pulmonary arterioles and venules was found significantly lower than systemic hematocrit.[26] Because the dynamic microhematocrit/systemic hematocrit ratio is known to decline with decreasing vessel diameter,[61] hypoxic constriction would be expected to cause a further reduction of dynamic microhematocrit in pulmonary microvessels. However, an increase of dynamic microhematocrit was observed during 1LV. The apparent discrepancy is most likely caused by the simultaneous reduction of RBC velocity, which diminishes the radial gradient of flow velocity and thus reduces the Fåhræus-Lindqvist effect as well as 'cell screening'.[62]

In summary, isoflurane has been shown by direct microscopic observation in rabbits to increase pulmonary arteriolar and venular diameters during 1LV. Thus isoflurane in concentrations used in clinical practice (0.75 minimum alveolar concentration) causes pulmonary vasodilation thereby counteracting the effect of HPV, increasing blood flow in the hypoxic lung, increasing $\dot{Q}_S/\dot{Q}_T$ and decreasing Pa_{O_2}.

The authors thank Professor Dr. Dr. h.c. Konrad Meßmer and Professor Dr. Dr. h.c. Klaus Peter for helpful suggestions and criticism, Silke Galland for technical assistance, and PD Dr. Fritz Krombach and Silvia Münzing for flow-cytometric measurements.

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