Hemodilution and hyperoxia locally change distribution of regional pulmonary perfusion in dogs

MARTIN KLEEN,1,2 OLIVER HABLER,1,2 JÖRG HUTTER,1 GREGOR KEMMING,2 ARMIN PODTSCHASKE,1 MATTHIAS TIEDE,1 MARTIN WELTE,2,3 PETER E. KEIPERT,3 SANJAY BATRA,3 N. SIMON FAITHFULL,3 CARLOS CORSO,1 BERNHARD ZWISSLER,2 AND KONRAD MESSMER1

1Institute for Surgical Research and 2Institute of Anesthesiology, Klinikum Grosshadern, University of Munich, 81366 Munich, Germany; and 3Alliance Pharmaceutical Corporation, San Diego, California

Kleen, Martin, Oliver Habler, Jörg Hutter, Gregor Kemming, Armin Podtschaske, Matthias Tiede, Martin Welte, Peter E. Keipert, Sanjay Batra, N. Simon Faithfull, Carlos Corso, Bernhard Zwissler, and Konrad Messmer. Hemodilution and hyperoxia locally change distribution of regional pulmonary perfusion in dogs. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H520–H528, 1998.—In seven anesthetized dogs, the effects of acute normovolemic hemodilution (ANH) to a hematocrit of 20 and 8% and the effects of hyperoxic ventilation (100% oxygen) on distribution of regional pulmonary blood flow (rPBF; radioactive microspheres) were investigated. Normovolemia was monitored with blood volume measurements (indocyanine green dilution kinetics). Before ANH, fractal dimension (D) of rPBF in the whole lung was 1.19 ± 0.09 (mean ± SD). Spatial correlation (r) of rPBF in the whole lung was 0.6 ± 0.08. D is a resolution-independent measure for global rPBF distribution, and r is the averaged flow relationship of directly neighboring lung samples. With regard to the entire lung, neither ANH nor hyperoxia changed D or r. With regard to horizontal, isogravitational planes, ANH induced opposite changes of rPBF heterogeneity depending on the vertical location of the plane and the parameter used. In ventral planes, a change in relative dispersion (SD/mean) indicated decreased homogeneity. However, r suggested more homogeneous perfusion. Hyperoxia restored baseline rPBF distribution. Our data suggest that ANH causes different alterations of heterogeneity of rPBF depending on location within the lung.

Acute normovolemic hemodilution (ANH) has been shown (32) to reduce the requirement for allogenic blood transfusion during elective surgery. Potential risks such as virus transmission and immunosuppression can thus be avoided or diminished (2). Increased clinical use of this method stimulated interest in examination of unresolved questions regarding regional blood flow characteristics during or after ANH.

High inspiratory oxygen fractions (FIO2) are often used in conjunction with ANH to compensate for the decreased oxygen-carrying capacity. However, hyperoxia has been shown to alter microvascular blood supply as assessed by means of multiwire PO2 electrodes and local hydrogen washout (23, 27), and the brain has been shown to exhibit disturbed tissue PO2 patterns when exposed to hyperoxia (12). Hypervolemic hemodilution has been shown to reduce fractal dimension (i.e., to homogenize perfusion) in the canine myocardium (9). These findings imply that hyperoxia and hemodilution can alter regional organ perfusion heterogeneity, which may be of significance in patients. This would become particularly important when, due to underlying disease, organ perfusion is disturbed before preoperative ANH. We investigated the effects of ANH alone and in combination with hyperoxia on regional pulmonary blood flow (rPBF).

Regional perfusion heterogeneity of the lung and other organs can be quantified using methods of fractal analysis (8, 15, 33). rPBF has also been demonstrated to be spatially correlated (13).

Spatial correlation of regional perfusion of hypoxic rabbit myocardium was found to be significantly different from that of the normoxic organ (29). On the basis of this finding, a change in this measure of perfusion heterogeneity induced by hemodilution and hyperoxia seemed likely.

Distribution of pulmonary blood flow is influenced by gravity (36), although gravity is much less important than originally suspected (14, 21). We therefore also studied the influence of gravity on distribution of rPBF.

We hypothesized that both ANH (by virtue of homogenizing perfusion) and hyperoxia (by disturbing microcirculatory regulation) would alter distribution of rPBF in opposite directions and that these effects would be independent of gravity. This hypothesis was tested with the use of fractal analysis, analysis of spatial correlation of rPBF, and relative dispersion of rPBF. Spatial correlation and relative dispersion were calculated globally and separately for isogravitational, 1.2-cm-thick planes of the lungs.

**METHODS**

**Animals**

This study was performed in seven beagle dogs of either sex fasted for 24 h before the experiments. Mean body weight was 17.2 ± 1.7 kg. All animals were splenectomized at least 8 wk before entering the study to avoid splenic contraction-induced changes of arterial oxygen content during ANH (34). The study was approved by the governmental animal care and use committee. All animals received care in compliance with the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892].

**Animal Preparation**

After intramuscular premedication with propiomazine (1.5 mg/kg body wt), anesthesia was induced by intravenous
injection of fentanyl (75 µg/kg body wt) and pancuronium bromide (0.1 mg/kg body wt) and maintained by inhalation of 0.8% isoflurane and continuous infusion of fentanyl (0.3 µg·kg body wt⁻¹·min⁻¹) and pancuronium bromide (2 mg/h). Fluid losses were replaced by intravenous infusion of Ringer solution at a rate of 1 ml·kg body wt⁻¹·h⁻¹. A warming pad was used to keep central body temperature above 37°C. After induction of anesthesia, animals were kept in the supine position for the duration of the protocol.

The animals were endotracheally intubated (8-mm inner diameter; HI-Lojet, Mallinckrodt, Argyle, NY) and mechanically ventilated on room air at a rate of 12 cycles/min with positive end-expiratory pressure of 7 cmH₂O (Servo 900B, Siemens-Elema, Solna, Sweden). Inspiratory time was set to 1.66 s, and tidal volume was adjusted to maintain normocapnia.

Arterial pressure was measured in the abdominal aorta with a 5-Fr micromanometer (PC-370, Millar Instruments, Houston, TX) introduced via the left femoral artery. Left ventricular pressure was measured with a 7-Fr micromanometer (SENTRON, Roden, The Netherlands) introduced into the left ventricle via the right carotid artery. A 7.5-Fr Swan-Ganz catheter (Edwards Swan-Ganz, Baxter Healthcare, Irvine, CA) was floated into the pulmonary artery via the left femoral vein for measurement of pulmonary artery pressure (PAP), right ventricular stroke volume, cardiac output, and central blood temperature. For injection of radioactive microspheres, a 5-Fr pigtail catheter (Cordis, Roden, The Netherlands) was introduced into the left ventricle via the left carotid artery. For injection of indocyanine green (ICG), an 8-Fr catheter (Arrow International, Reading, PA) was placed in the superior vena cava. Arterial blood sampling and blood withdrawal for blood volume measurements were performed with an 8.5-Fr catheter introducer sheath (Arrow International) in the left femoral artery. All vessels were surgically exposed and identified before insertion of catheters. Catheter positions were verified by fluoroscopy and pressure readings.

Further catheters were inserted but not used for producing the data reported in this manuscript: a balloon catheter (8/22-Fr Fogarty occlusion catheter, Baxter Healthcare) allowing occlusion of the inferior vena cava and a venous sampling catheter (5-Fr, Cordis) in the coronary sinus.

Experimental Design

The present report deals with part of a comprehensive experimental study involving 22 dogs that was designed to assess the ability of a perfluorocarbon (PFC)-based oxygen carrier to support tissue oxygenation and myocardial function during profound hemodilution (see Ref. 19 for previous results).

All animals were identically instrumented with various catheters (see Animal Preparation). Initially, all animals were ventilated with an FIO₂ of 0.21 and isovolemically diluted with 6% hydroxyethyl starch (HES; mol wt 200,000, substitution ratio 0.45–0.55; Fresenius, Bad Homburg, Germany) to a hematocrit of 20 ± 1%. Subsequently, the FIO₂ was increased to 1.0. All animals were then randomly assigned to one of three groups and received either PFC emulsion or placebo before continued normovolemic hemodilution. Regional pulmonary perfusion was assessed only in the group of animals that did not receive PFC.

In this study, we report the rPBF data for seven animals undergoing the initial hemodilution (ANH1 0.21), exposure to 100% oxygen (ANH1 1.0), and further dilution to a hemoglobin concentration of 3 g/dl (ANH2 1.0).

Blood volume was determined before hemodynamic measurements were made. If blood volume was below the control or preceding value, an appropriate amount of 6% HES was given. On completion of the protocol, the animals were euthanized by intraventricular injection of saturated potassium chloride solution and passively exsanguinated. The lungs were removed for dissection and subsequent assessment of distribution of regional blood flow.

Measurements

Blood volume. Blood volume (BV) was measured by determining the dilution kinetics after injection of ICG (CardioGreen, Becton-Dickinson Microbiology Systems, Cockeysville, MD) using the “whole blood” method (7). Through the central venous catheter, 0.25 mg/kg body wt of ICG was injected. Arterial blood was drawn through a transparent cuvette attached to a densitometer in a closed system, and light absorption was measured for a period of 7 min. Every 30 s, absorption values were read and the blood was reinjected. With a three-point calibration line constructed immediately before each dye injection, absorption values were converted into ICG concentrations. Linear extrapolation of the resulting time-concentration elimination curve on a semilogarithmic coordinate system to the time point of dye injection yielded the theoretical dye plasma concentration at injection time (C₀). BV was then calculated as BV = DI/C₀, where DI is the injected ICG dose.

Hemodynamic measurements. PAP was recorded using a Statham P23Db transducer positioned at midthorax height. Thermolodized cardiac output and right ventricular stroke volume were measured in triplicate at end expiration and averaged. Cardiac index and right ventricular stroke volume index were calculated using body surface area (BSA) determined according to Holt et al. (22): BSA = k·BW²/³, where BW is body weight and k = 11.2.

Blood samples. Arterial and mixed venous blood gases were determined using an ABL-300 blood gas analyzer (Radiometer, Copenhagen, Denmark). Hemoglobin concentration, arterial and mixed venous oxygen content, and saturation were measured by absorbance spectrophotometry (682 CO-Oximeter, Instrumentation Laboratory, Lexington, MA).

rPBF. rPBF was determined with a modification of the radioactive microsphere method previously shown (26) to yield results of high precision and low bias compared with the conventional technique. Briefly, microspheres were injected into a systemic circuit and were trapped in pulmonary capillary vasculature after arteriovenous shunting. Hence, perfusion of all organs including the lung could be determined by means of a single injection of microspheres.

For each measurement, 7.2 ± 2.1 × 10⁸ microspheres (diameter 15.5 ± 0.5 µm; NEN-Trac, DuPont, Wilmington, DE) labeled with a randomly selected radioisotope (¹⁴¹Ce, ⁵¹Cr, ¹¹⁵In, ⁹⁵Nb, ⁴⁶Sc, or ⁸⁵Sr) were suspended in 0.9% saline to a total volume of 5 ml. The amounts of microspheres for each nuclide were adjusted to equalize emitted radiation and minimize methodological error with a computer program (24). For injection, microspheres were transferred into glass vials and agitated for a minimum of 3 min. Injection was then performed over a period of 50 s into the left ventricle. During injection, the vial was continuously agitated to prevent settling of the microspheres. No cardiorespiratory changes were noted after injection of microspheres.

After the animals were euthanized, their lungs were removed and freed from hilar vessels and adipose and lymphatic tissue and then dried for 3 days with a continuous positive airway pressure of 25 cmH₂O. The lungs were then suspended in a rigid [1.5-cm polyvinylchloride (PVC) board], plastic-lined box and embedded in polyurethane (PU) foam (Assil, Henkel, Düsseldorf, Germany). After the PU was polymerized, the lungs within the PU block were divided into
samples (n = 710 ± 180 for both lungs of one animal). First, the block was cut into 1.2-cm-thick slices. The dimensions of the slices were standardized with a distance-restricting board attached to the PVC box. The slices were then divided into 1.2-cm³ tissue cubes with the use of a PVC board with molded grooves designed to guide a specially designed scalpel grip. Tissue samples were assigned three-dimensional coordinates. Pieces mostly consisting of airways were excluded before weighing and documenting coordinates. Before weighing and determination of radioactivity, all adhering PU foam and all bronchi and vessels greater than ~1.5 mm in diameter were removed.

Tissue sample radioactivity was counted for 300 s using a 1,024-channel gamma counter (model 5650, Packard Instruments, Downers Grove, IL) with a 3-in. NaI (Tl) crystal detector connected to a multichannel analyzer (series 35plus MCA, Canberra Industries, Meriden, CT). Specific software (MIC-III) (18) allowed for separation of the gamma spectra, half-life correction, and data management. Samples weighing <9 mg were excluded to minimize weighing errors. The weight-normalized number of microspheres in each sample was used as a measure for relative blood flow.

Fractal analysis. A commonly used approach for analyzing the fractal nature of pulmonary perfusion is to dissect the lung into small parts and measure the perfusion on this level. Flow data from these samples are then recombined with data processing software to obtain information about perfusion of larger samples.

If the perfusion is fractal, the natural logarithm of the increasing heterogeneity is linearly related to the natural logarithm of the decreasing sample size. Therefore, the heterogeneity of a fractal perfusion depends on the scale of measurement. The higher the resolution of the measurement, the more heterogeneous the fractal perfusion appears.

Heterogeneity of blood flow to the lung is described with the relative dispersion (RD; SD/mean) of regional perfusion. The scale of the applied measurement is expressed as the relative volume of the recombined tissue samples (V) in relation to a reference tissue sample volume (V0). Heterogeneity and scale of measurement of RPBF have been shown to be related, obeying the power law (RD·V)/(RD·V0) = (V/V0)1-D (17), where D is the fractal dimension of the perfusion. The slope of the regression line through multiple data points on a plot of RD-V vs. V/V0 is 1 - D. Such plots are therefore used to determine fractal properties of perfusion of the lung (8, 15, 33).

Data handling for fractal analysis of RPBF was performed with validated software developed at our institution (25). For calculation of D, individual samples as well as recombinations to larger tissue units comprising 4 (2 × 2), 8 (2 × 2 × 2), 12 (2 × 2 × 2), and 27 (3 × 3 × 3) individual samples were used. Recombinations were achieved by adding the microsphere contents of the samples comprised in an aggregate sample and dividing the result by the sum of the weights of the samples. D was calculated for the whole lung and separately for the dorsal and ventral halves of the lungs. Thickness isogravitational slices did not contain sufficient tissue pieces to allow for calculation of D for each plane.

Spatial correlation. In 1992, Glenny (13) suggested spatial correlation (p) of regional pulmonary perfusion as a new parameter for characterizing distribution of RPBF. For calculation of p, all blood flow values separated by a given distance are treated as data pairs and are used as variables for a three-dimensional extension of the standard correlation formula as reported by Glenny (13). We implemented this formula with a computer program (Watcom C/C++ compiler v.10; Powdersoft, Concord, MA). For analysis, we used the correlation values of samples separated by the smallest possible distance of 1.2 cm (1,2).

We calculated p separately for the whole lung (pglobal) and separately for four dorsal and four ventral horizontally oriented isogravitational planes (p_isograv).

Statistics

All statistical analyses were performed using SAS v.6.1 (SAS Institute, Cary, NC). Normal distribution of data was verified using the Shapiro-Wilks test. Analysis of variance for repeated measurements was used to test the effect of the variable "time point in the protocol" on distribution of dependent variables. If a significant influence of "time point" was elicited, Student-Newman-Keuls testing for paired comparisons was performed post hoc. The type 1 error level was set at 5%. Data are presented as means ± SD.

RESULTS

Cardiorespiratory parameters are presented in Table 1. Heart rate, arterial pressure, cardiac index, and stroke volume index increased only slightly during hemodilution from a hematocrit (Hct) of 36% to an Hct of 20%. Ventilation with 100% oxygen did not induce significant changes in these parameters. After ANH to an Hct of 8%, increased heart rate and right ventricular stroke volume produced a 76% enhancement of cardiac output, whereas arterial pressure dropped by 29%. To compensate for the reduced oxygen-carrying capacity after ANH to an Hct of 20%, the oxygen extraction ratio rose by 68% (from 20 to 32%). When the animals were ventilated with 100% oxygen, this was only partly reversed. On further hemodilution to an Hct of 8%, oxygen delivery was maintained by increased cardiac index alone, without any further increase in oxygen delivery.

| Table 1. Cardiorespiratory variables |
|---|---|---|---|
| Ctrl | ANH1 0.21 | ANH1 1.0 | ANH2 1.0 |
| Hct, % | 36 ± 3 | 20 ± 1* | 20 ± 1 | 8 ± 1* |
| HR, beats/min | 96 ± 14 | 104 ± 12 | 95 ± 12 | 112 ± 14* |
| MAP, mmHg | 107 ± 11 | 114 ± 8 | 109 ± 10 | 84 ± 25* |
| CI, l/min·m⁻² | 3.9 ± 1.0 | 4.6 ± 0.8 | 3.7 ± 0.9 | 6.2 ± 1.2* |
| SI, ml/m² | 41 ± 8 | 45 ± 7 | 39 ± 6 | 53 ± 8* |
| O₂XTR, % | 20 ± 5 | 32 ± 4* | 29 ± 7 | 35 ± 12 |
| SVRI, dyn·s·cm⁻⁵·m⁻² | 2,237 ± 595 | 1,940 ± 352 | 2,328 ± 509 | 1.100 ± 230* |
| LVEDP, mmHg | 7 ± 2 | 13 ± 3* | 14 ± 2 | 16 ± 5 |
| PAP, mmHg | 13.5 ± 3.5 | 17.4 ± 4.7 | 17.4 ± 3.6 | 19.0 ± 4.6 |
| BVI, ml/kg | 85 ± 11 | 81 ± 7 | 77 ± 6 | 82 ± 9 |
| PVR, mmHg | 46 ± 3 | 39 ± 2* | 57 ± 5* | 54 ± 9 |
| PaO₂, mmHg | 89 ± 8 | 93 ± 7 | 514 ± 19* | 520 ± 24 |
| PaCO₂, mmHg | 38 ± 4 | 38 ± 6 | 40 ± 3 | 38 ± 6 |

Values are means ± SD. Hct, hematocrit; HR, heart rate; MAP, mean arterial pressure; CI, cardiac index; SI, stroke volume index; O₂XTR, oxygen extraction ratio; SVRI, systemic vascular resistance index; LVEDP, left ventricular end-diastolic pressure; PAP, mean pulmonary artery pressure; BVI, blood volume index; PaO₂, mixed venous oxygen partial pressure; PaCO₂, arterial oxygen partial pressure; PaCO₂, arterial carbon dioxide partial pressure; Ctrl, baseline measurement; ANH1 0.21, measurement after hemodilution to Hct 20% at room air; ANH1 1.0, measurement after switch of FIO₂ to 1.0 at Hct 20%; ANH2 1.0, measurement after hemodilution to Hct 8% at FIO₂ 1.0. *P < 0.05 vs. previous time point in protocol (Student-Newman-Keuls test after significant result by analysis of variance).
extraction. Systemic vascular resistance index was reduced at both levels of hemodilution. Left ventricular preload (end-diastolic pressure) increased with hemodilution to an Hct of 20% and did not change subsequently. Pulmonary artery pressure was stable throughout the protocol. Isovolumic exchange of blood for HES preserved normovolemia as evidenced by unchanged blood volume indexes. The drop in mixed venous PO2 (PvO2) indicated a decrease in global oxygenation status after ANH to 20% that was reversed on administration of oxygen. No further change in PvO2 occurred on ANH to an Hct of 8%. The animals were adequately ventilated as reflected by constant PCO2 values. Pulmonary gas exchange remained unaffected during the protocol as shown by mean arterial PO2-to-FiO2 ratios of 419, 448, 510, and 514 mmHg.

Dog 3 died during measurement ANH2 1.0 at an Hct of 8% because of cardiocirculatory decompensation, as indicated by a sudden rise of left ventricular end-diastolic pressure and heart rate while arterial pressure fell suddenly. Up to this point, this animal was hemodynamically stable and no differences were demonstrable compared with the other animals. The data from the first three measurements for this animal were therefore excluded in the analyses.

Relative Dispersion

Relative dispersion of rPBF calculated for the entire lung (RDglobal), irrespective of gravitational planes, is shown in Fig. 1. There was no significant change in the average RDglobal during the protocol (Fig. 1A). Conversely, the relative dispersion of rPBF calculated separately for horizontal isogravitational slices of the lung (RDisograv) displayed a heterogeneous pattern (Fig. 1B). In all four dorsal isogravitational planes and in the most dependent ventral plane, there was no influence of either intervention. In the second ventral plane, however, there was a tendency toward greater heterogeneity after ANH (P = 0.08), and in the two uppermost ventral planes, there was a significantly more heterogeneous perfusion pattern after ANH (P = 0.01 and 0.03, respectively). After hyperoxia was induced, these changes were completely reversed. Hemodilution to an Hct of 8% did not further influence RDisograv. The ventral slices in which changes of RDisograv had taken place comprised 26% of the total lung tissue mass analyzed.

Fractal Dimension

Global fractal dimensions (D) of regional perfusion from all animals are shown in Fig. 2A. The correlation coefficient r of the natural logarithm of RD and the natural logarithm of V/V0, averaged over all animals at all time points was -0.97 ± 0.02. At baseline, D was 1.19 ± 0.09. Initial hemodilution to an Hct of 20%, hyperoxia, or further hemodilution to an Hct of 8% had no significant effects on this parameter. D for all lung tissue samples vertically situated above and below a midthorax line (i.e., the ventral and dorsal halves of the lungs) were calculated separately (Fig. 2B). There were no significant differences between D from different time points or between D from dorsal and ventral compartments of the lungs.

Spatial Correlation

Correlations of perfusion to adjacent lung samples calculated for the whole lung (Dglobal), irrespective of gravitational planes, are shown in Fig. 3A. No significant effect of either intervention on Dglobal was seen. Hemodilution to an Hct of 20%, hyperoxia, or hemodilution to an Hct of 8% had no significant effects on this parameter.

For calculation of D for isogravitational planes (Disograv), the two most dependent and two uppermost slices were each combined into one slice to obtain larger numbers of samples. Similar to RDisograv, Disograv indicated unchanged heterogeneity in all dorsal and in the first ventral plane. Conversely, in contrast to RDisograv, in the two uppermost ventral planes, ANH induced a tendency of homogenization in the second to last plane.
values in the ventral parts, whereas hyperoxia completely restored the prehemodilution pattern. Hemodilution to an Hct of 8% did not affect this distribution pattern.

DISCUSSION

Main Finding

The main finding of this study is that ANH induces a change in heterogeneity of rPBF only in the nondependent parts of the lung and that this change is completely reversed by hyperoxia.

Model

The alterations of the macrohemodynamic parameters observed on hemodilution in the present study are in agreement with what is known for intentional hemodilution of anesthetized animals (31). We conclude that our canine model may be used for the study of the effects of ANH.

The microsphere technique was chosen for assessment of rPBF because it is an accurate method specifically validated for pulmonary perfusion measurements (30) and has been used extensively for similar studies of rPBF (8, 13, 33). The modification of the original

(P = 0.07) and a significant homogenization in the uppermost plane (Fig. 3B).

In Fig. 4A, \( r \) is plotted as a function of distance between paired tissue samples for one exemplary animal. The typical pattern of decaying \( r \) with increasing distance was seen in all animals. Histograms of relative blood flow to the ventral halves of the lung from one animal are shown in Fig. 4B. A tendency toward heterogenization after ANH and reversal of this effect after switch to 100% oxygen can be seen in these histograms. The homogenization after ANH to an Hct of 8% in this animal was not consistently seen in other dogs. This is also reflected by the scatter of \( R_{D_{iso grav}} \) and \( R_{D_{iso grav}} \) for the ventral parts of the lungs after the second ANH (Figs. 1B and 3B).

Relative Flow

The relative blood flow to horizontal planes was calculated as mean specific microsphere content (SMC) in one plane divided by mean SMC of the whole lung. Specific microsphere content is defined as the number of microspheres per gram of tissue. The most dependent parts of the lung consistently exhibited less relative flow than the midregions (Fig. 5). Hemodilution to an Hct of 20% induced a tendency toward increased flow in the dependent parts but reduced relative flow

Fig. 2. Fractal dimension (D) of rPBF at protocol time points Ctrl, ANH1 0.21, ANH1 1.0, and ANH2. A: global D. B: D of ventral and dorsal halves of lung. Values are means ± SD.

Fig. 3. Spatial correlation of rPBF to neighboring tissue cubes at protocol time points Ctrl, ANH1 0.21, ANH1 1.0, and ANH2. A: global spatial correlation. B: spatial correlation of rPBF to neighboring tissue cubes calculated separately for isogravitational, 1.2-cm-thick slices. * \( P = 0.04 \), baseline vs. ANH1 0.21 in combined slices ventral 3 and 4.
The technique we used for this study was developed and validated in our laboratory (26).

**D and RD of rPBF**

The D of rPBF allows measurement of the increments in apparent heterogeneity of rPBF with increasing resolution of the measurement. Thus D permits comparison of data from different research groups. D of rPBF determined with RD can vary between 1, indicating deterministic distribution of rPBF, and 1.5, when perfusion is random (6).

Values of D observed in this study at baseline were not different from those reported in previous reports (5, 8, 33) in dogs and sheep. Conversely, Glenny and
Robertson (16) reported lower D (i.e., greater homogeneity) of between 1.04 and 1.10 of lungs from anesthetized dogs.

At baseline, RD of rPBF in our experiments was similar (30.2 ± 4.2%; sample size 1.7 cm³) to that reported by Walther and colleagues (35) in sheep (29.5 ± 6.5%; sample size 2 cm³). However, others found higher values. Parker et al. (33) reported 47.3% in awake standing dogs (sample size 1 cm³). In supine anesthetized dogs, Glenny and Robertson (16) found RD of 50% (sample size 1.9 cm³), and Melsom et al. (30) observed 38% in awake goats. Using sample sizes of 8 cm³, Caruthers and Harris calculated RD of 64% in isolated sheep lungs that were positioned analogous to the supine position of a sheep (8).

Differences in RD are due in part to different sample size and to our practice of removing all airway structures and vessels >1.5 mm in diameter from pulmonary tissue samples. Walther et al. (35) excluded samples with >25% airway content and observed RD similar to what we observed. Because bronchi are perfused systemically, bronchial perfusion may contribute to measurement error with respect to rPBF. By removing the nonpulmonary tissue from the lung samples, more homogeneous lung tissue content is obtained. Therefore, a more homogeneous perfusion and smaller RD are expected, but D of rPBF should not be affected because D is derived from the change in RD upon change of measurement scale. This theoretical assumption was confirmed by results we obtained for D that are consistent with previous reports (8, 33).

$\rho$ of rPBF

The $\rho$ of adjacent tissue samples gives additional information concerning distribution of rPBF in comparison to RD and D. RD averages the perfusion of a larger volume of the lung (e.g., the whole lung, a lobe, or a slice), whereas $\rho$ averages mutual relationships of flow to paired tissue blocks separated by a chosen distance, in our case 1.2 cm (i.e., the distance between two neighboring tissue blocks). Matsumoto et al. (29) recently reported that $\rho$ of regional myocardial perfusion is increased by hypoxia compared with normoxic ventilation. At baseline, we obtained a mean spatial correlation coefficient for a 1.2-cm distance between samples $[\rho_r(1.2)]$ of 0.60 ± 0.06. Glenny reported a $\rho_r(1.2)$ of 0.72 ± 0.07 in supine dogs, indicating more homogeneous rPBF (13), in contrast to a later study in which higher RD reflected more heterogeneous rPBF (16) than was found in the present study. There is also a discrepancy in D between the data of Glenny and Robertson (16) and that of our study (see D and RD of rPBF). Our findings, however, are in agreement with D of rPBF given by Parker et al. (33) and Caruthers and Harris (8). Therefore, we assume that the relatively small difference in $\rho_r(1.2)$ reflects a small true difference in perfusion heterogeneity.

Changes of Distribution of rPBF

The effects of hemodilution and hyperoxia on D, RD, and $\rho$ of rPBF have not been assessed previously. None of the interventions exerted a significant effect on distribution of global rPBF. Assessment of heterogeneity parameters for the whole lung is the technique used by most investigators. Conversely, our data show that large alterations in distribution of rPBF on hemodilution and hyperoxia may be present or absent in lung tissue depending on the vertical location of the sample within the lung. The changes in rPBF distribution induced by hemodilution (Hct 20%) in the ventral parts of the lung were reversed by hyperoxia, indicating opposite effects on distribution of rPBF. This is consistent with studies in which high FIO2 was found to cause more heterogeneous local tissue Po2 values in brain and skeletal muscle (12, 23, 27).

Furthermore, we observed a striking difference between heterogeneity change assessed with RD compared with $\rho_{sograv}$. Hemodilution induced an increase in RD (i.e., an increase of heterogeneity) in ventral parts of the lungs, whereas $\rho_{sograv}$ of ventral slices increased with ANH, which indicates homogenization of rPBF. This apparent difference between RD and $\rho$ has also been observed by Glenny and Robertson (16). They found rPBF to be more homogeneous in prone than in supine dogs when assessed with RD but more heterogeneous when assessed with $\rho$ (16). Quantitatively, RD, $sograv$ fell to the same extent as RD, from 45.8 ± 14.8 to 40.8 ± 9.6%. We attribute this seemingly paradoxical result to the global nature of RD as opposed to the local nature of $\rho$. For calculation of RD, the standard deviation of perfusion to one (normally large) lung region is divided by the averaged perfusion of this region; thus RD is by definition global. On the other hand, $\rho$ averages the linear relationship of blood flow to many sample pairs in the lung region separated by a given distance; thus $\rho$ is an average of local perfusion relationships. $\rho$ is therefore more than just an additional parameter of heterogeneity of rPBF but rather a parameter of a different kind of heterogeneity, probably best described as averaged local heterogeneity.
Profound hemodilution to an Hct of 8% at an F\textsubscript{1\textsubscript{O}}\textsubscript{2} of 1.0 elicited no changes in any of the parameters of heterogeneity. We had expected that at an Hct of 8%, the changes induced by moderate hemodilution to an Hct of 20% would be enhanced. Because hyperoxia abolished all ANH-induced effects, we speculate that when 100% oxygen was inspired, a very strong effect opposing the ANH-induced effects was produced that could not be overcome by more severe hemodilution.

Mechanisms Affecting D and \rho

Recently, it has been shown that systemic vasodilation and enhancement of cardiac output upon hemodilution are partly influenced by the \textit{L}-arginine-nitric oxide pathway in anesthetized rats (11). Increased activity of nitric oxide synthase due to hemodilution might allow redistribution of blood flow to less perfused lung regions through dilatation of supplying vessels, thus homogenizing blood flow.

Cardiac output is usually higher after hemodilution, resulting in homogenization of global pulmonary perfusion and reduction of venous admixture (10). However, this mechanism does not influence microperfusion heterogeneity, which is described by D and \rho. Perfusion heterogeneity of small (4 × 4 × 11 mm) lung cubes remained unaffected by changes of cardiac output (20). The authors found recruitment of vessels in the upper regions of the lung but no change in dependent and nondependent perfusion gradients.

In the ventral planes of the lung, where global heterogenization (RD; Fig. 1) prevailed after ANH, there may have been a tendency toward flow reduction even though there was no significant effect on relative flow in these regions (Fig. 5). Reduced flow might result in inhomogeneous derecruitment of vessels, which per se would increase heterogeneity of rPBF. This, however, contrasts with increased \rho in ventral planes upon ANH (Fig. 3). It could be speculated that derecruitment occurred in a regional manner that would result in increased heterogeneity when slices are viewed in their entirety with RD but that would leave the average relationship of closely neighboring tissue blocks (\rho) unaffected.

The exact mechanisms by which ANH and hyperoxia regionally change homogeneity of rPBF remain to be clarified.

Limitations of Present Study

In general, bronchial blood flow is low and does not contribute much to pulmonary perfusion (1, 3). When shunted microspheres are used, however, microspheres reaching the lung via the bronchial circulation may falsely increase calculated rPBF because the concentration of radioactive microspheres in arterial blood feeding the bronchial arteries is much higher than in venous blood containing recirculating microspheres. Therefore, bronchial arterial blood flow will deposit more microspheres per milliliter than pulmonary artery blood flow. We did not sample mixed venous blood during microsphere injection and could therefore not calculate systemic shunt. If systemic shunt decreases, the relation of microsphere concentration in bronchial artery blood and microsphere concentration in mixed venous blood will become high, thus increasing the error imposed on our method by the bronchial circulation. In dogs, bronchial blood amounts to <1% of cardiac output (4, 28), and we have observed average systemic shunt of >10% in dogs (26). These figures compute to 10–60 times more microspheres reaching the lung via the pulmonary artery than through bronchial circulation. This relation is further increased in favor of the pulmonary circulation by the fact that only 55% of bronchial blood flow reaches the pulmonary parenchyma (4). We have removed all airways with diameters >1.5 mm, thereby removing a great proportion of microspheres delivered by the bronchial arteries.

In conclusion, our data demonstrate spatially divergent effects of ANH and hyperoxia on heterogeneity of rPBF in anesthetized, healthy dogs. In contrast to our hypothesis, hemodilution rendered rPBF more heterogeneous in ventral isogravitational planes (RD\textsubscript{isograv}). The local heterogeneity, however, as assessed with \rho, indicated homogenization of rPBF in ventral isogravitational planes. The biological significance of these changes as well as the mechanisms involved remain to be elucidated.

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Address for reprint requests: M. Kleen, Institute for Surgical Research, Klinikum Grosshadern, Univ. of Munich, Marchioninistr. 15, 81366 Munich, Germany.

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