Spatial pattern of pulmonary blood flow distribution is stable over days

ROBB W. GLENNY, STEVEN MCKINNEY, AND H. THOMAS ROBERTSON
Departments of Medicine and of Physiology and Biophysics,
University of Washington, Seattle, Washington 98195

Glenny, Robb W., Steven McKinney, and H. Thomas Robertson. Spatial pattern of pulmonary blood flow distribution is stable over days. J. Appl. Physiol. 82(3): 902–907, 1997.—Despite the heterogeneous distribution of regional pulmonary perfusion over space, local perfusion remains stable over short time periods (20–100 min). The purpose of this study was to determine whether the spatial distribution of pulmonary perfusion remains stable over longer time periods (1–5 days). Regional blood flow was measured each day for 5 days in five awake standing dogs. Fluorescent microspheres of different colors were injected into a limb vein once for 5 consecutive days in standing awake dogs. Fluorescent microspheres (4) were used to mark regional pulmonary blood flow each day for 5 consecutive days in standing awake dogs.

METHODS

Experimental protocol. The study was approved by the University of Washington Animal Care Committee. Five mongrel dogs (weight 25.2–26.0 kg) of either gender were studied. While standing on four paws, each animal had an 18-gauge butterfly needle placed in a superficial leg vein. Fluorescent 15-µm-diameter microspheres (FluoSpheres, Molecular Probes, Eugene, OR) of five different colors (blue-green, orange, yellow-green, red, and crimson) were injected intravenously over 30 s in 5 ml of saline and were followed by a 10-ml saline flush. The microspheres were sonicated and vortexed before injection. Microspheres of one color were injected each morning on five consecutive mornings. Two million microspheres of the first four colors and 4 million crimson microspheres were injected in varying order. After each microsphere injection, the butterfly needle was removed.

After the microsphere injection on the final day, each animal was anesthetized with thiopental sodium. Femoral vein and arterial catheters were inserted, and the animals were deeply anesthetized, given heparin, exsanguinated, and killed by intravenous pentobarbital sodium. A sternotomy was performed, large-bore catheters were placed in the pulmonary artery and left atrium, and the thoracic aorta was clamped off. The lungs were perfused with normal saline and 2% dextran (mol wt 74,000) until clear of blood, removed from the chest, and allowed to dry inflated at an airway pressure of 25 cmH2O.

When dry, the lungs were coated with Kwik Foam (DAP, Dayton, OH), suspended vertically in a plastic-lined square box, and embedded in rapidly setting urethane foam (2 lb. Polyol and Isocyanate, International Sales, Seattle, WA) to create a rigid form to which a three-dimensional coordinate system could be applied. The foam block was cut into uniform sized cubes that were ~1.9 cm3 in volume. Foam adhering to the lung pieces was removed. Each lung piece was weighed and assigned a three-dimensional coordinate and lobe designation.

Spatial pattern of pulmonary blood flow distribution is heterogeneously distributed in space when observed at any instant in time (1, 7, 8, 12–14). A recent study has also shown that regional perfusion varies over 20- to 100-min intervals (6). Although statistically significant, the variability in blood flow over time is relatively small compared with the total heterogeneity of perfusion. Because high-flow regions remain high flow and low-flow regions remain low flow, regional blood flow is highly correlated from one time point to another over short time intervals (6). The small variability in local blood flow is not random over time, and the neighboring pieces have similar patterns of blood flow changes (6). These findings can be interpreted as supporting the concept that blood flow is distributed by an asymmetrical branching vascular tree that produces heterogeneous flow in space. The temporal variability in local blood flow occurs about a mean flow for each piece determined by the vascular tree and may be attributed to vasomotion at the arterial and arteriolar level (6).

Because of the relatively short time intervals used in the previous study (6), temporal blood flow variability occurring on longer time scales may have been overlooked. It is possible that regional perfusion may vary enough so that the mean flow to regions becomes more uniform when averaged over time (10). In addition, mechanical ventilation and anesthesia used in prior studies (6) may have affected the spatial and temporal variability of blood flow.

The goal of this study was to determine the temporal variability of regional pulmonary blood flow over a longer time period in awake and spontaneously breathing dogs. To this end, fluorescent microspheres (4) were used to mark regional pulmonary blood flow each day for 5 consecutive days in standing awake dogs.
The fluorescent signals for each color were determined by extracting the fluorescent dyes from each piece with an organic solvent and then measuring the concentration of fluorescence in each sample (4). Relative blood flow to each lung piece was calculated by dividing the measured fluorescence of each piece by the mean fluorescence of all pieces for that color. The data set for each dog consisted of x, y, and z coordinates; lobe designation; weight; and relative flow for each lung piece. The relative flow to each lung piece was divided by the weight of each lung piece and normalized to the mean, providing a relative weight-normalized flow per piece.

Statistical analysis. Relative weight-normalized flows are used for all analyses and are hereafter referred to simply as flow or perfusion. Values are means ± SD. Pearson’s correlation coefficient r calculated between perfusion to lung pieces within a dog is used to quantify the relationship between regional perfusion on different days. The coefficient of variation (CV) was used to characterize perfusion heterogeneity within each dog over space or time.

We used the method of Iversen (9) and assumed that the methodological noise can be estimated by the Poisson distribution if errors due to fluorescent measurements are minimized. When performed properly, the methodological noise from the fluorescent measurements is insignificant, accounting for <0.1% of the observed variability in flow (4). The methodological error in each lung piece (CV method i) over time is estimated from the multiple time points by the equation

\[ CV_{\text{method } i} = 1 / \bar{r}_i \]

where \( \bar{r}_i \) is the mean number of microspheres in each piece i over the 5-day period. The number of microspheres of a given color in each lung piece is estimated by multiplying the number of microspheres injected by the fraction of flow to each lung piece (fluorescent signal of one color/sum of all number of microspheres injected by the fraction of flow to color in each lung piece) is estimated by multiplying the over the 5-day period. Then the number of microspheres of a given relative flow of 1.0, resulting in a CV method of ~2.6% for such a piece.

The microsphere injection method provides a measure of blood flow distribution at a “snapshot” in time (10). In reality, the microspheres provide an average flow value to each lung piece over the 30-s injection time (or longer, due to slurring of the concentration-time curve for the entering microspheres) and cannot measure any temporal variation in flow during this time. Our measure of temporal heterogeneity, therefore, ignores any variability over the injection interval, and we define the temporal heterogeneity of flow for each piece (CV temporal j) as

\[ CV_{\text{temporal } j} = \sqrt{CV_{\text{piece } i}^2 - CV_{\text{method } i}^2} \]

where CV piece i is the CV over time for each piece i. A measure of the temporal variability for an animal (CV temporal j) is obtained by averaging all pieces across CV temporal j for that animal. The spatial variability for each animal on a given day (CV spatial j) is determined from the CV across the estimated flows to all pieces on day j. Because temporal heterogeneity of perfusion is ignored over the microsphere infusion time, CV spatial j does not require adjustment for temporal variability.

Noise in the observed spatial heterogeneity is insignificant because of the large numbers of pieces and microspheres per piece. An estimate of the error for a distribution that has a true CV of 30% with 1,400 pieces that have an average of 1,500 microspheres per piece is 0.4%. A single measure of spatial variability for an animal (CV spatial i) is obtained by averaging CV spatial j across all days for that animal.

A statistical procedure based on “runs” is used to determine whether the temporal variability in regional perfusion is random over time (6). In this test, the difference between flow at a given time and the mean flow (a residual) is determined, and the number of times the residual for a given piece changes sign is tabulated for all pieces. A permutation test uses the experimental data from each animal but randomly shuffles the flow measurements in time within each piece and recounts the number of runs. A probability distribution for the total number of runs is estimated by randomly permuting the data set for each animal a large number of times and by calculating the number of runs for each trial. The observed temporal pattern is not considered random if the observed total number of runs for the animal occurs in the 5% tail area (P < 0.05) of the probability distribution determined from the permutation trials. Mean occurrences and the 95% confidence intervals for a given flow pattern are approximated from the binomial distribution, with the probability for a specific pattern being defined by its frequency in the permutation trials.

Least squares linear regression is used to characterize the vertical gradient of blood flow by determining the slope of regional flow as a function of distance up the lung from the ventral surface. Analysis of variance is used to identify the most important determinants of regional blood flow variability over time and to compare differences in correlation coefficients between different time intervals.

Spatially, an aberrant large orange fluorescent signal occurs in isolated lung pieces. The cause of this erroneous signal has not yet been identified. These aberrant signals can be identified by visual inspection of the fluorescent values. One animal in this study (dog 1) was noted to have five lung pieces with exceptionally high fluorescent orange signals, identified as outliers because their fluorescent values exceeded six SDs from the mean. These five pieces were excluded from the data set before analyses. The other animals had no lung pieces with fluorescent signals exceeding three SDs from the mean for any color (including orange).

**RESULTS**

**Spatial heterogeneity.** The number of lung pieces obtained from each animal and their mean CV spatial are

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>No. of Lung Pieces</th>
<th>CV spatial, %</th>
<th>CV temporal, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,354</td>
<td>27.0</td>
<td>7.7</td>
</tr>
<tr>
<td>2</td>
<td>1,473</td>
<td>30.3</td>
<td>6.9</td>
</tr>
<tr>
<td>3</td>
<td>1,296</td>
<td>30.7</td>
<td>6.8</td>
</tr>
<tr>
<td>4</td>
<td>1,430</td>
<td>27.8</td>
<td>5.7</td>
</tr>
<tr>
<td>5</td>
<td>1,487</td>
<td>31.6</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Mean ± SD 29.5 ± 2.0 6.9 ± 0.7

CV spatial, spatial coefficient of variation (see text); CV temporal, temporal coefficient of variation (see text).
presented in Table 1. CV\textsubscript{spatial} averages 29.5% and ranges from 27.0 to 31.6%. When examined by lobe, no consistent difference across animals exists in the spatial variability of flow among lobes. The vertical gradient of blood flow is not different from zero across all animals.

Temporal stability. Regional perfusion is very stable over time. High-flow pieces remain high flow and low-flow pieces remain low flow (Fig. 1). The correlations among flows to each piece between different days are shown in Table 2. The correlation coefficients among flows between different days average 0.930. When the correlation coefficients are grouped by the number of days between injections, no difference between the groups when using analysis of variance is observed.

Temporal heterogeneity and patterns of temporal variability. Temporal variability in regional flow as measured by CV\textsubscript{temporal} ranges from 5.7 to 7.7%, with an average of 6.9%. Values for all dogs are presented in Table 1.

A characteristic of temporal heterogeneity apparent in three of the animals is that low-flow regions have the greatest CV\textsubscript{temporal} (Fig. 2). CV\textsubscript{temporal} is corrected by using Eq. 2 for the methodological noise caused by the small number of microspheres depositing in low-flow regions.

None of the spatial coordinates (x, y, or z) adds significantly to the determination of CV\textsubscript{temporal} after incorporating the mean flow to each piece. When examined by lobe, CV\textsubscript{temporal} differs across lobes within an animal, but lobes with the greatest variability are not consistent among animals.

Fluctuations in regional flow are not random over time. The permutation test based on runs (Fig. 3) shows that the total number of runs is significantly different from that expected by chance (P < 0.05) in all animals except dog 5. The temporal pattern of blood flow in this dog may, in fact, not be random, but a pattern could not be detected with only five time points. Three of the animals have patterns of flow variability with fewer runs than expected by chance, and one dog has a pattern with more runs than expected by chance. The pattern of flow is different than chance because of flow redistribution that occurs less than expected or because blood flow tends to change more frequently than expected. Figure 4 shows the frequency distribution of runs within a dog and from the permutation test of the same animal. There are more pieces with only two runs (crosses mean only one time) in the observed data set compared with the expected, demonstrating that the pattern of blood flow over time in this animal is more stable than expected.

The temporal variability in flow can be explored in finer detail by examining the patterns of change within each piece. The flow pattern within a piece having two runs can change sign at one of four times (e.g., between days 1 and 2, 2 and 3, 3 and 4, or 4 and 5). The patterns of change can be symbolized by an X representing a change in sign and an O representing no change.

### Table 2. Correlation among flows to each piece between different days

<table>
<thead>
<tr>
<th>Interval Between Injections, days</th>
<th>r</th>
<th>No. of Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.931 ± 0.028</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>0.934 ± 0.024</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>0.922 ± 0.028</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>0.934 ± 0.028</td>
<td>5</td>
</tr>
<tr>
<td>Mean</td>
<td>0.930 ± 0.006</td>
<td>50</td>
</tr>
</tbody>
</table>

Correlation values are means ± SD.

Fig. 1. Regional blood flow to all lung pieces within 1 animal on 2 different days. Despite long interval of 4 days between flow determinations, blood flow to each piece on different days is highly correlated. N, no. of lung pieces.

Fig. 2. Relationship between temporal heterogeneity and flow per piece (dog 2). Most of variability in flow over time occurs in low-flow pieces. Calculated coefficient of variation (CV) due to temporal heterogeneity (CV\textsubscript{temporal}) has been corrected for method noise. N, no. of lung pieces.
the example of a piece with a single change in sign over the 5 days, four different patterns exist, XOOO, OXOO, OOXO, and OOOX. A pattern represented by OXOX has sign changes between days 2 and 3 and between days 4 and 5. Because we do not indicate whether the change is from positive to negative or from negative to positive, there are a total of 15 possible patterns. The frequency distributions of these patterns for one dog are shown in Fig. 5.

In this animal, most of the variability in flow that accounts for the large numbers of pieces having only one change in sign (2 runs) is due to a sign change between days 3 and 4. All of the dogs having blood flow that is more stable than expected by chance have a similar pattern, with change occurring primarily on 1 day. The one dog with more variability in flow than expected had a pattern where sign changes occurred every day.

DISCUSSION

The principal finding of this study is that in normal awake dogs regional pulmonary perfusion is spatially heterogeneous and the pattern of distribution remains very stable over days. Long-term studies such as this one are now more feasible with the use of fluorescent microspheres, obviating the need for radioactivity.

Our conclusions are limited to the temporal and spatial resolution of our methods. Because the microsphere method provides an average flow value to each lung piece over the 30-s injection time, any temporal variation in flow during this time is not measured. Similarly, temporal variability within lung pieces, such as alveolar capillary flow heterogeneity (16), cannot be observed.

The spatial heterogeneity of regional pulmonary blood flow is significantly lower in this study than in previous study, in which the same size lung pieces in prone dogs were used (5). The primary differences between our prior experiments and this study are that...
the dogs were not anesthetized, not mechanically ventilated, and were standing on four paws. Using slightly smaller pieces, Parker and associates (14) reported the spatial heterogeneity of pulmonary perfusion in awake standing dogs to be 47 ± 12%. When the fractal relationship between piece size and spatial heterogeneity was used to determine the perfusion heterogeneity at the piece size used in the present study, dogs used by Parker and co-workers had a CV of 43 ± 12%. This is not statistically different from the spatial heterogeneity observed in the present study. Recent studies by Walther and co-workers (15) demonstrated that pulmonary perfusion becomes more heterogeneous during anesthesia and mechanical ventilation in standing/prone sheep. They demonstrated that the CV spatial increases from 25.9 to 28.1% with pentobarbital sodium anesthesia and increases further to 33.4% when the animals are mechanically ventilated. General anesthesia may directly or indirectly alter the distribution of pulmonary perfusion. Whereas inhaled anesthetics can dampen hypoxic pulmonary vasoconstriction (HPV) (3), the intravenous anesthetics used in our prior studies (pentobarbital, ketamine, and valium) did not affect HPV significantly (3). Alternatively or additively, anesthetic agents may alter regional ventilation and indirectly affect blood flow distribution through HPV (3). Mechanical ventilation may alter blood flow distribution via its effects on regional ventilation and, therefore, alter blood flow by either HPV or the mechanical influence of alveolar pressure on regional blood flow.

Regional pulmonary blood flow in awake dogs remains stable across days. High-flow pieces are always high flow, and low-flow pieces are always low flow. The $r^2$ between days averages 0.865. Hence, 87% of the variability in piece flow is explained by the variability in regional flow on another day. The strongest determinant of flow to any given piece is the flow to that piece at another time point, suggesting that the mechanism(s) determining regional blood flow is (are) relatively fixed over time in a lung free of pathology. Whereas regional perfusion can vary about a local mean, the displacement from this mean is limited. Correlation of regional flows is, therefore, very strong, regardless of the time interval between observations.

The temporal variability of regional perfusion is lower in awake standing dogs than in anesthetized and mechanically ventilated dogs. Our previous study (6) exploring the temporal variability of regional pulmonary blood flow documented a CV temporal of 17.2 ± 7.6% compared with a CV temporal of 6.9 ± 0.7% in this study. This difference may again be attributable to the lack of anesthesia and mechanical ventilation in the present study. A second potential explanation is that the longer microsphere infusion times used in this study (30 vs. 20 s) could mask some of the very rapid temporal variation occurring during infusion. Iversen (9) used microspheres to explore the temporal variability of myocardial blood flow by varying both the microsphere infusion times and intervals between injections. He used a 10-s or 5-, 10-, or 30-min infusion time and either a 1- or 15-min interval between infusions. He found that the correlation between blood flow to a region at two times increased as the microsphere infusion time increased and concluded that whereas regional coronary vasomotion is characterized by a wide range of cycle times, cycle times <5 and 10 min are more prominent. Because our primary interest in this study is the temporal fluctuations in regional perfusion over time intervals of 1-5 days, the microsphere infusion time of 30 s is meant to be short enough to mark blood flow distribution at a snapshot in time while still representing the distribution of perfusion over a few respiratory cycles. The marked reduction in temporal variability can be attributed to the longer microsphere infusion time only if the cycle time of the temporal variability is close to 30 s. Cycle times of <10 s will be equally smoothed by 20- and 30-s infusion times, and cycle times of 60 s or greater will not be significantly smoothed by either infusion time. Although we do not know to what extent a 30-s microsphere infusion time dampens temporal fluctuations compared with a 20-s infusion, it seems unlikely that the marked reduction in the temporal variability of blood flow between the two studies can be accounted for by the small differences in microsphere infusion times. The primary difference between the two studies is the use of awake spontaneously breathing animals in the present study.

Despite the long time interval between microsphere injections, the temporal variations in regional perfusion are not random. The test of runs demonstrates that the temporal pattern of blood flow over 5 days significantly differs from chance in four of the five animals. Three of these animals had fewer observed runs than its associated permutation test, indicating that regional flow was positively correlated with the flow to the same region on adjacent days. The one dog with more observed runs than its associated permutation test demonstrates regional perfusion that correlates negatively with the flow to the same region on adjacent days. Oscillations in local perfusion could explain the patterns observed. If local perfusion does oscillate, the cycle times have to be on the order of a few days, because cycle times <1 day will appear random when sampled at a frequency of one each day. We believe that a simple sinusoidal pattern is unlikely and that a more likely scenario is for local perfusion to be stable for days, reacting temporarily to some local influence and then returning to its baseline flow. If the reaction to local stimuli resolves within a day, blood flow will not appear random when sampled at daily intervals.

Because the spatial distribution of pulmonary blood flow cannot be explained by a hydrostatic gradient (5), other mechanism(s) must be responsible for the heterogeneous distribution. One potential mechanism is the geometry of the pulmonary vascular tree (7). Asymmetric distribution of blood flow at vascular bifurcations can produce heterogeneous blood flow. Local factors may influence flow so that perfusion varies slightly over time but remains strongly correlated from one time to another. It appears that the factors controlling
regional perfusion at the local level play a small role in determining perfusion distribution. Alternatively, a basal tone determined by local mediators may be present that remains very stable over days (2). The vasoactive control of pulmonary blood flow distribution plays a much greater role in disease conditions.

Address for reprint requests: R. W. Glenny, Division of Pulmonary and Critical Care Medicine, Univ. of Washington, Box 356522, Seattle, WA 98195 (E-mail: glenny@pele.pulmcc.washington.edu).

Received 29 May 1996; accepted in final form 6 November 1996.

REFERENCES