Hypoxia-Induced Acute Changes in Capillary and Fiber Density and Capillary Red Cell Distribution in the Rat Heart

Friedrich Vetterlein, Hartmut Hemeling, Jürgen Sammler, Attila Pethó, and Gerhard Schmidt

The influence of acute hypoxia (respiration gas 12%, 10%, and 8% O₂ and asphyxia, respectively) on 1) the density of perfused capillaries and muscle fibers, and 2) the capillary red cell distribution was investigated in the left heart of anesthetized rats. To observe capillaries and fibers, fluorescein isothiocyanate-labeled (FITC)-γ-globulin and lissamine-rhodamine-B200-labeled (RB200) myoglobin were injected intravenously as labels of the perfused intravascular and the extracellular space, respectively. The hypoxic conditions were induced subsequently and maintained for 3 minutes. After this period the heart was rapidly frozen for histological demonstration of the dyes. Ventilation with 12% or 10% O₂ did not induce any changes in the density of perfused capillaries; however, 8% O₂ in respiration gas did lead to a significant increase (capillaries/mm²: subepicardium, 4,180, controls, 3,620; subendocardium, 3,930, controls, 3,240). A similar increase was found in the asphyxia group (capillaries/mm²: subepicardium, 4,170; subendocardium, 3,700). The increases in the density of perfused capillaries were paralleled by rises in fiber density. This leads to the conclusion that the changes in capillary counts were caused by fiber elongation with a resultant decrease in intercapillary distances. This assumption was supported by observations that there were no signs of changes in ventricular segment length during respiration of 12% or 10% O₂ but that an increase did occur with 8% oxygen and with asphyxia. Densities of perfused capillaries exactly coincided with anatomical densities (demonstrated by additional labeling of capillary basement membranes with isolectin B₄) in normoxic and asphyctic hearts. The distribution of red cells in the capillaries, determined in histological sections, did not differ appreciably under hypoxia due to reduced O₂ in respiration air (12%) or asphyxia. The results obtained indicate that 1) extreme hypoxic states cause the capillaries to move closer to each other due to elongation of myocardial fibers and 2) red cell distribution is not altered during these conditions. (Circulation Research 1989;64:742-752)
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Distances. Comparisons of distances between perfused capillaries necessitate a constant geometry of the anatomical capillary network. This, in turn, is basically linked with a constancy of fiber thickness. Fiber dimensions, however, may change under certain conditions, for example, high doses of epinephrine or propranolol. These substances have been found to cause fiber elongation and thereby force the capillaries to move more closely together. It appears reasonable to assume that such a mechanism might also play a role during hypoxic states since dilation of the ventricular chambers was also demonstrated in severely hypoxic hearts. It is the aim of this study to analyze whether such a mechanism might influence capillary densities during states of oxygen deficiency. Since plasma and red-cell-perfused capillaries may differ with respect to their flow characteristics, densities of plasma perfused capillaries as well as their red cell content should be established.

Materials and Methods

General Procedure

The experiments were performed on male Wistar rats of 90–130 g body weight. The animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The body temperature was kept constant at 37–37.5°C by a heating device (Yellow Springs Instrument Co., Yellow Springs, Ohio). Catheters were placed into the femoral vein for injection of drugs and into the femoral artery for measurement of the arterial blood pressure (Statham P23Db), which was continuously monitored on a direct writing system (Gould Brush Inc., Cleveland, Ohio). The heart rate was derived from the pressure wave and digitally indicated on a beat to beat counter. This method was unsuccessful during asphyxia-induced hypotension. During this condition, the frequency could still be determined through measurement of the ventricular segment lengths (see below).

A tube was placed into the trachea, and the animal was artificially ventilated using a positive-pressure rodent respirator (type 681, Harvard Apparatus, South Natick, Massachusetts). The animals inhaled a gas mixture of 25% oxygen and 75% nitrous oxide. The thoracic cavity was opened by transsection of the third to fifth left ribs. The exposed heart was continuously moistened with Ringer's solution at 37°C. In part of the experiments, the left auricle was cannulated and the tip of the catheter placed into the left atrium. Hypoxic conditions were induced in two different ways:

1) The animals were changed to a gas mixture consisting of 12%, 10%, or 8% O₂ and 75% N₂O in N₂. Two minutes later an arterial blood sample (50 μl) was drawn and analyzed for its Po₂ and PcO₂. Functional morphologic parameters of the cardiac microcirculation (see below) were determined by quickly freezing the heart 3 minutes after onset of hypoxic conditions.

2) The respirator was turned off and microcirculatory parameters were determined after 30 seconds and 3 minutes, respectively. In one experimental set, asphyxia for 30 seconds was followed by reoxygenation for 2 minutes; this was repeated three times. In the control experiments, Po₂ and PcO₂ were also controlled shortly before freezing the heart. In all experiments the respiratory volume was adjusted to attain a control Po₂ of 80–100 mm Hg.

Experimental Group I: Measurement of Density of Plasma-Perfused Capillaries

Fluorescein isothiocyanate (FITC)-labeled γ-globulin (0.3 ml/100 g body wt) and lissamine rhodamine B 200 (RB200)-labeled myoglobin (0.5 ml/100 g body wt) were slowly injected 12 minutes before onset of hypoxia/asphyxia. At the end of the period of oxygen deficiency, the heart was clamped at its base, rapidly transferred into isopentane at −100°C for about 10 seconds, and then stored in liquid nitrogen. Frozen sections of the heart were prepared by cutting at right angles to the heart axis at the largest circumference of the organ. In six to 10 areas of the subendocardium and subepicardium, the density of both FITC-labeled capillaries and fibers was counted using a ×100 oil-immersion objective and a ×10 eyepiece. The latter were visible as a result of the labeling of the extracellular space with RB200. As a criterion for counting, care was taken so that within the counting field the cross sections of the muscle fibers did not show any preferential orientation. Evaluated subepicardial fields were localized in tissue one or two layers below the external surface. (For further details of the procedure described above see Vetterlein and Schmidt.)

Experimental Group II: Measurement of Myocardial Segment Length In Vivo

To determine changes in myocardial fiber geometry that might indirectly influence capillary density, changes in myocardial segment length was monitored during the different intensities of systemic hypoxia. Segment lengths were considered to be an index of the degree of ventricular chamber dilation and this, in turn, an index of fiber elongation.

For this purpose, a device was designed that made it possible to record continuously the distance between two points on the ventricular surface (Figure 1). The measuring system consisted of two thin metallic arms hinged together at the top. At the peripheral end, they were attached to the cardiac surface with histoacrylic cement. In the angle just below the apex, a light pipe was mounted at each branch in such a way that the facing ends of the pipes could shift parallel to each other. In this way, an increase in the distance between the attached points was transformed into a signal characterized by decreasing intensity of light transmitted across
Densities Determined by Basement Membrane Staining Versus Plasma Labeling

Experimental Group III: Comparison of Capillary In all, 12 experiments were performed in this group. the end of the first and the beginning of the second.

interval of at least 10 minutes was maintained between hypoxic conditions were tested in each animal. An

Mean distances between these points were evaluated at intervals of 10 seconds and expressed in percent of the length obtained during the control period. In this experimental group the effects of two hypoxic conditions were tested in each animal. An interval of at least 10 minutes was maintained between the end of the first and the beginning of the second. In all, 12 experiments were performed in this group.

Experimental Group III: Comparison of Capillary Densities Determined by Basement Membrane Staining Versus Plasma Labeling

In this group, RB200-labeled γ-globulin (0.2 ml/100 g body wt) was injected intravenously, and 15 minutes later, the heart was removed as described above. Only two states, normoxia and 30 seconds of asphyxia, were tested. These are the conditions that proved to induce the greatest variations in the degree of ventricular dilation. The frozen sections were treated as follows: The tissue slices were again taken up on frozen alcohol, transferred to room temperature and then, in contrast to the former procedure, kept in this solution for 10 minutes. This period of time was necessary for a subsequent gradual transfer of the slices into the aqueous phase without loss of the intravasal globulins. For observation of the vascular basement membrane, isolec tin B4 from Bandeiraea simplicifolia, conjugated with FITC (Sigma Chemie, Deisenhofen, FRG) was then applied at a concentration of 0.07 mg/ml for 15 minutes.20 In contrast to the technique of Peters and Goldstein,20 solution of isolec tin and subsequent washing of the slices (15 minutes) was performed in carbonate-buffer solution (pH 9.0). The slices were gradually retransferred into alcohol and finally embedded in Entellan.

Evaluation of the slices was performed in the following way: In a section taken from the largest circumference of the heart in the subepicardium and subendocardium, FITC fluorescence was used to count the cross-sectioned capillaries that were visible due to the accumulation of the dye in the vascular basement membrane. After drawing the respective vessels on the paper with the aid of the camera lucida, the filters were changed to show the RB200 fluorescence of the labeled globulin, which could then be compared in its distribution with that of the capillary wall (Figure 4).

In this series, only comparative data were obtained because the procedure of tissue processing (immersion in pure water) proved to induce significant shrinkage, which would have led to artificially high absolute counts.

Experimental Group IV: Measurement of Capillary Red Cell Content

The red cell content was established by determining the length of cell-free sections in capillaries. To make the vascular system visible, FITC-labeled γ-globulin (see above) was injected 1 minute before induction of the hypoxic conditions. Since it has been suggested that the red cell content is higher in rapidly perfused capillaries than in low flow channels, this aspect was also dealt with by labeling the rapidly perfused capillaries with a second dye. Therefore, RB200-labeled γ-globulin was infused into the left atrium 1 second before removal of the heart. In this way, all the capillaries, including those labeled within 1 second, could be distinguished in the histological sections. Freeze sectioning and fluorescence microscopy were performed as described earlier. In these experiments, the sections were cut parallel to the heart axis so that the ventral and the dorsal part of the left ventricle became visible. The red cell content was deter-
Capillary and muscular fibres and on other physiological variables.

**Table 1. Effect of Hypoxia on Density of Perfused Capillaries and Muscular Fibers and on Other Physiological Variables**

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hypoxia for 3 minutes</th>
<th>Asphyxia</th>
<th>3x Asphyxia (30 sec.)</th>
<th>reoxygenation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=15)</td>
<td>12% O₂ (n=8)</td>
<td>10% O₂</td>
<td>8% O₂ (n=8)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>Arterial P₀₂ (mm Hg)</td>
<td>94.7±2.4</td>
<td>44.7±3.1</td>
<td>25.2±1.8</td>
<td>16.0±1.8</td>
<td>35.2±3.8</td>
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<tr>
<td>Arterial P₀₂ (mm Hg)</td>
<td>39.4±1.8</td>
<td>34.0±3.1</td>
<td>25.3±3.0</td>
<td>19.3±1.5*</td>
<td>47.0±2.9</td>
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<tr>
<td>Mean arterial blood</td>
<td>105±6</td>
<td>37±4*</td>
<td>87±11</td>
<td>65±12</td>
<td>34±1*</td>
</tr>
<tr>
<td>pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15±4*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>403±12</td>
<td>394±20</td>
<td>388±24</td>
<td>253±24*</td>
<td>390±6</td>
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<tr>
<td>Cap/mm², subepi</td>
<td>3,600±130</td>
<td>3,730±230</td>
<td>3,760±200</td>
<td>4,180±280*</td>
<td>4,570±240*</td>
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<td>Cap/mm², subendo</td>
<td>3,240±140</td>
<td>3,290±220</td>
<td>2,950±70</td>
<td>3,930±190*</td>
<td>4,040±220*</td>
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<tr>
<td>Fib/mm², subepi</td>
<td>3,370±200</td>
<td>3,780±190</td>
<td>3,710±130</td>
<td>4,880±340*</td>
<td>4,610±270*</td>
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<tr>
<td>Fib/mm², subendo</td>
<td>3,310±240</td>
<td>3,440±150</td>
<td>3,450±200</td>
<td>4,380±430</td>
<td>4,680±290*</td>
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<tr>
<td>Cap/Fib ratio, subepi</td>
<td>1.01±0.05</td>
<td>0.99±0.02</td>
<td>1.03±0.07</td>
<td>0.89±0.02</td>
<td>1.01±0.03</td>
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<tr>
<td>Cap/Fib ratio, subendo</td>
<td>0.95±0.06</td>
<td>0.90±0.02</td>
<td>0.88±0.06</td>
<td>0.92±0.06</td>
<td>0.88±0.04</td>
</tr>
<tr>
<td>Left ventricular</td>
<td>11.4±2.6</td>
<td>7.9±2.5</td>
<td>8.4±1.0</td>
<td>24.8±4.5*</td>
<td>30.5±1.5*</td>
</tr>
<tr>
<td>lumen,% of section</td>
<td>area</td>
<td></td>
<td></td>
<td></td>
<td>27.0±3.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Cap, capillaries; Fib, fibers; subepi, subepicardium; subendo, subendocardium.

*Significant difference versus control group (U test).

†Value not determinable.

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mined in the longitudinally sectioned areas of the subepicardium and the subendocardium. For contrast enhancement of the red cells, the benzidine technique was applied. The original method was somewhat modified (reduction of water content in the solution) to keep from impeding the fluorescence localization in the tissue. Benzidine (500 mg) was dissolved in 12.5 ml absolute ethanol. To this solution 0.5 ml H₂O₂ (30%) was added, followed by 4.5 ml of 70% ethanol. A few drops of this final solution were transferred onto a precooled (liquid nitrogen) glass slide; the tissue section was then placed upon the frozen solution. When the thawing ethanol had penetrated the slice, the slide was kept at room temperature for 5 minutes. The benzidine-H₂O₂ solution was then replaced by absolute ethanol followed by the embedding medium (Entellan).

In the final few experiments, it was discovered that the demonstration of red cells could be simplified by using transmitted light of 450 nm without any prior staining process. The light was scattered by placing a semitransparent foil below the slide. In this way, the contrast within the tissue was greatly reduced, with the exception of the red cells; they could be clearly distinguished as blue corpuscles.

In every experiment three slices, each of which lay at least 100 μm from the next, were evaluated; in each slice, six areas in the subepicardium and six areas in the subendocardium were chosen. Evaluations were made in areas where capillaries ran parallel to the sectioning plane and where part of them contained RB200. A ×40 objective and a ×10 eye-piece were used. With the aid of a camera lucida the length of the capillaries was measured by drawing them onto a sheet of paper; RB200-labeled capillaries were indicated separately. The light was then changed from incident to transmitted to evaluate the red cell content. Since in part of the vessels single cells overlapped each other and thus could not be counted separately, only the fraction of plasma gaps per evaluated capillary length was evaluated. Seven to 30 lengths were counted in each of these areas.

**Statistical Analysis**

All data are given as means±SEM. For comparison of the data the U test was used. The data of Group IV were analyzed by a two-way variance analysis. This procedure was used separately for both the subepicardial values and the subendocardial data. For comparison of the distribution curves the Kolmogoroff-Smirnoff test was applied.

**Results**

In the final evaluation, only those experiments were used in which the mean arterial blood pressure exceeded 80 mm Hg in the normoxic control period.

**Experimental Group I: Density of Plasma-Perfused Capillaries and Muscular Fibers**

The basal circulatory data are presented in Table 1. In the control group mean arterial blood pressure was 105 mm Hg, the heart rate was 403 beats/min, and the pressure of arterial blood gases was 94.7 mm Hg Pₐ and 39.4 mm Hg Pco₂ immediately before freezing the heart. Reduction of respiratory oxygen content to 12% led to a decrease in blood pressure (37 mm Hg) and a slight decrease in heart rate (394 beats/min). When the oxygen content of the respiration gas was further reduced (10% and 8% O₂), blood pressure decreased at first, but then began to rise again to a mean level of 87 and 65 mm Hg, respectively. Asphyxia due to respiratory arrest led to a rapidly developing hypotension within 30 seconds (34 mm Hg). After 3 minutes of asphyxia, a...
value of 15±3 mm Hg was attained; the heart rate could not be determined from the blood pressure curve at this point in time.

The results of capillary and fiber countings showed that when the respiration gas was changed, resulting in an arterial Po2 of 45 or 30 mm Hg, no differences in capillary density could be observed as compared with the controls. However, when the respiration gas was reduced to 8% O2 (arterial Po2 16.0 mm Hg), an increase in capillary as well as fiber density occurred (capillaries/mm², controls: subepicardium, 3,620; subendocardium, 3,240; hypoxia, arterial Po2 16 mm Hg: subepicardium, 4,180; subendocardium, 3,930). The experiments involving respiratory arrest also revealed densities of fluorochrome-labeled capillaries significantly higher than those of the controls: subepicardium, 4,170; subendocardium, 3,700 capillaries/mm².

The increases in density of perfused capillaries were correlated to changes in fiber density compared with the normoxic controls. No significant difference in fiber density could be found in the 12% and 10% O2 groups. With 8% O2 in respiration gas as well as with asphyxia, fiber densities significantly increased. When calculating capillary-to-fiber ratios, this fraction was relatively constant in the various experimental groups (Table 1).

The changes in density of perfused capillaries and of muscle fibers were found not only to be correlated with the level of arterial Po2 attained during the 3-minute period but also to depend on the speed of onset of hypoxia. When respiration was entirely interrupted (asphyxia), arterial Po2 was 35 mm Hg after 30 seconds. At this point, the increase in capillary and fiber density was already highly significant; however, no such change was
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Figure 3. Mean changes in arterial blood pressure (MAP), heart rate (HR), and segment length (SL) in anesthetized rats subjected to different degrees of O2 deficiency in respiration gas and to asphyxia. Mean±SEM, each group n=6. △, Hypoxia, 12% O2; □, hypoxia, 10% O2; ○, hypoxia, 8% O2; ●, asphyxia.

noted when this level was gradually reached by changing the O2 content of respiration gas to 10%.

The changes in capillary and fiber density were entirely reversible. When short periods of asphyxia and reoxygenation were induced, no effects on the final capillary and fiber densities were observed (Table 1).

Experimental Group II: Myocardial Segment Lengths In Vivo

To confirm a hypoxia-induced increase in the density of perfused capillaries caused by fiber elongation, this experimental series was used to test whether a dilation of the myocardial wall as an index of fiber lengthening may be directly observed in an in vivo preparation without freezing the heart.

The changes in arterial blood pressure, heart rate, and segment lengths observed in this group are shown in Figures 2 and 3. When the animals inhaled a gas mixture of reduced oxygen content (12%, 10%, or 8% O2), the blood pressure in all groups first declined within 30 seconds but then stabilized or even increased again with continuation of hypoxia, depending on the degree of O2 deficiency. Blood gases in these three groups were (3-minute value) PO2 42.4±2.3, 32.9±1.4, 19.4±0.9 mm Hg; PCO2 22.2±3.1, 21.4±2.7, 12.3±1.3 mm Hg, respectively. Heart rate was unaffected in the 12% and 10% O2 groups; only the 8% O2 group showed a decrease during the second and third minute of hypoxia. The most intensive effects on blood pressure and heart rate were observed in the asphyxia group. Blood pressure began to fall within 10 seconds, and heart rate began to decline within 30 seconds. When asphyxia did not exceed 30 seconds, the effects on blood pressure, heart rate, and segment length (see below) were entirely reversible (Figure 2). With the continuation of respiratory arrest, however, extreme bradycardic periods occurred, alternating with phases of acceleration. The latter, however, never exceeded prehypoxic values. As a rule, pump failure developed within 3 minutes, and restitution of respiration did not restore cardiac function.

Segment lengths showed the following changes (Figures 2 and 3): A slight decrease in segment length occurred in the 12% O2 group, meaning that contractility had somewhat increased. No change in this parameter was found in the 10% O2 group, and a protracted increase in segment lengths occurred in the rats ventilated with 8% O2.

An abrupt increase in segment length was observed at asphyxia (Figure 2). This increase took place immediately after artificial ventilation was stopped; in fact, it usually even preceded the decline in blood pressure. In most cases the length value returned to preasphyxia values within about 10 seconds. Then, while blood pressure began to fall, the length value increased again and reached its highest levels within 1 minute. With continuation of asphyxia, this parameter showed a slight tendency toward lower values. Identical effects were observed when the animals were ventilated with an oxygen-free gas mixture instead of interrupting artificial ventilation.

Experimental Group III: Comparison of Capillary Densities Determined by Basement Membrane Staining Versus Plasma Labeling

The preceding experiments have shown that extreme hypoxia or asphyxia leads to cardiac chamber dilation, fiber elongation, and a decrease in intercapillary distances. To further prove the assumption that these changes in cardiac geometry have indeed caused the observed increases in density of plasma-filled capillaries, the latter parameter was compared with a direct index of morphological density (basement membrane staining) in additional experiments. In six control experiments (PO2 98.0±6.5; PCO2 30.4±0.8 mm Hg), as well as in six asphyxia (30 seconds) experiments (PO2 30.0±2.5; PCO2 39.9±6.7 mm Hg), a 100% congruity was found between capillaries identified by lectin-labeling and by filling with globulin (Figure 4). This means that changes in morphological rather than functional density had indeed induced the observed increases.

Experimental Group IV: Capillary Red Cell Content

In the experiments in which changes in capillary red cell content were studied, the following basal data were obtained. In the control group, the arterial blood pressure was measured at 84 ± 5/68 ± 4 mm Hg, the heart rate at 376 ± 9 beats/min, and the arterial PO\textsubscript{2} at 91 ± 1.8 and PCO\textsubscript{2} at 37.7 ± 2.5 mm Hg. The corresponding data for the animals ventilated with 12% O\textsubscript{2} were arterial blood pressure 37 ± 2 mm Hg, heart rate 448 beats/min, and arterial PO\textsubscript{2} 42.4 ± 0.7 and PCO\textsubscript{2} 34.1 ± 1.4 mm Hg. (Each group, n=8). In those animals in which ventilation had been stopped for 3 minutes, the blood pressure was lower than 20 mm Hg. The heart rate and blood gases could not be determined in this group.

The results of the measurement of the red cell content are summarized in Table 2. These data express the length of capillaries free of red cells (plasma gaps) as a percentage. The means of this parameter were similar in all experimental groups. No statistically significant difference (two-way analysis of variance) was found when the subepicardial and subendocardial values were compared. Within each group, the percentage of cell-free sections was higher in the subendocardial region than in the subepicardial. However, only in those animals that had been subjected to respiratory arrest did the difference become statistically significant.

The question arose whether major differences between the normoxic and hypoxic hearts might nevertheless exist when the frequency distribution of the single values (cell-free fraction within each capillary) are considered. Frequency distribution curves of this parameter are shown in Figure 5. Despite the small shifts of the mean values (described above), no differences were established when the data of the three experimental groups were compared. In all groups, extremely low as well as extremely high values of red cell filling were found. The distribution curves did not show any significant differences when the control

<table>
<thead>
<tr>
<th>Percent (mean) of capillary lengths without red cells</th>
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<tbody>
<tr>
<td>Subepicardium</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Normoxia</td>
</tr>
<tr>
<td>Hypoxia</td>
</tr>
<tr>
<td>Asphyxia</td>
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</tbody>
</table>

In the mean, 300 capillaries were evaluated per experiment. Each group, n=8.
curve was compared with the corresponding subepicardial or subendocardial curve of the hypoxia or asphyxia group (Kolmogorov-Smirnov test).

Subsequently, we investigated whether the intensity of capillary red cell filling reflects differences in capillary flow rate. To determine this, RB200-globulin was also infused for just one second before freezing the heart. Capillaries that had been reached by this dye were considered to have been perfused at a higher rate than those stained solely with FITC. In three control experiments and in six experiments with reduced oxygen content in the respiration gas, areas found in the tissue were sufficient for such a comparison. The fraction of plasma gaps proved to be nearly identical in the control experiments for capillaries labeled with one or both dyes (mean percentage of capillary lengths without red cells, capillaries labeled with FITC only: 41±7%; with RB200 and with FITC: 42±6%). In the hypoxia-experiments, the fraction of cell-free segments in the capillaries also proved to be identical for this parameter with a slight, but still insignificant, tendency toward an increase in the percentage of plasma gaps (mean percentage of capillary length without red cells, capillaries labeled with FITC only: 41±6%; with RB 200 and with FITC: 46±4%). Thus, no proof was found that might account for a higher red cell filling in capillaries perfused at a higher rate.

Discussion

The present experiments revealed that an acute increase in anatomical capillary density results from elongation of myocardial fibers during extreme hypoxia. No significant changes in capillary plasma gaps were found. These results are based both on findings that showed an increase in absolute fiber density, an unchanged capillary-to-fiber ratio, increases in segment lengths, and an unchanged ratio of functional versus anatomical capillary density and on measurements of plasma gaps in hearts of rats subjected to different degrees of oxygen deficiency.

The question that arises first is whether methodological artifacts might have influenced these findings. It is imperative that the actual geometry of the heart be maintained during the process of removal and freezing. Clamping of the heart base should keep the lumina from emptying; this is essential because muscles rapidly contract when immersed in a cold environment.23 Because of the high heart rate in rats, it was not possible to distinguish between systolic or diastolic phases in these experiments. Since clamping was carried out completely at random, all phases of the cardiac cycle are probably represented. Nevertheless, the results strengthen the view that the freezing technique does make it possible to characterize the mean contractile state of the heart by random sampling. In the hypoxia experiments, reduction in respiratory oxygen content resulting in a P02 of up to 30 mm Hg had no significant effects on fiber density or segment length. However, extreme reduction in respiratory O2 content and asphyxia did induce a measurable increase in fiber density. This was paralleled by the increase in segment length in vivo.

The latter technique of determining changes in ventricular segment length proved a difficult problem due to the small size of the heart in young rats. Because of the short absolute distances to be measured, the usual and most reliable techniques (cineangiocardiology and implantation of ultrasonic transducers24) could not be applied. For this reason, an especially small-scale photoelectric system capable of implanting such a label onto the surface of the rat ventricle was designed for the present study. The weight of the system was 80 mg. The pressure

![Figure 5. Frequency distribution of the percentage of capillary lengths in the rat heart containing no red cells; effect of hypoxia (12% O2 for 3 minutes) and asphyxia (interruption of artificial ventilation for 3 minutes). Each group, n=8.](http://circres.ahajournals.org/doi/abs/10.1161/01.CIR.60.3.749?journalCode=circ)
exerted on the surface of the heart was reduced even further through the elastic light pipes fastened above the animal.

Demonstration of the anatomical wall of the myocardial capillaries by binding the fluorochrome-labeled isoelectin B4 with the capillary basement membrane proved a highly valuable method. Examination of the microvascular system in histological sections showed that no interruption in staining occurs at the venous or arterial site of the microvascular system. Decreased staining at the venous end is a problem inherent to the alkaline-phosphatase technique. This method, first proposed by Romanul and Bannister25 and since used by many authors to determine anatomical capillary density, was also incompatible with the simultaneous demonstration of fluorochrome-labeled globulins distributed intravascularly.

To measure the capillary red cell content, the fraction of cell-free lengths was ascertained. This mode of evaluation was used because the number of cells could not be counted exactly in all capillaries due to the overlapping of cells whenever intracapillary hematocrit was very high.

Changes in red cell distribution might have occurred during the process of removal and freezing of the heart. The same considerations hold true here as described with regard to the distribution of the plasma labels.14 The fact that the dye, when injected for extremely short periods of time (1 or 2 seconds), is indeed found in only a portion of the capillaries strengthens the view that the intracoronary blood does not become mixed with nonlabeled blood during this critical phase. The observation that in the histological preparations the intracapillary red cells had, in part, maintained a shape similar to that seen in vivo may be taken as additional proof that no gross alterations in cell distribution occurred during removal and fixation of the organ.

Any interpretation of the present results should take into consideration previous studies that have shown that a plasma label reaches all capillaries available during normoxic conditions within 5 seconds. In addition, the present study has shown that even periods of asphyxia do not open up additional, previously unperfused channels. With reoxygenation, capillary and fiber densities were unchanged as compared with the controls. Channels that opened only during phases of asphyxia would have also maintained the plasma label in the following normoxic period and would have become visible.

Additional proof for the latter view is given by the verified presence of capillary basement membranes and the intravasal plasma label within the same section. No difference in density could be found at all in normoxic or asphyxic hearts.

Therefore, to explain the increased functional capillary density observed by other authors during hypoxia, it appears essential to postulate that there is a mechanism involved other than the opening up of capillaries previously unperfused with plasma for more than a few seconds.

One possibility is the elongation of myocardial fibers, resulting in a reduction of the intercapillary distances. This mechanism has also been demonstrated in previous experiments on the effects of, for example, coronary occlusion.16 The present study has clearly shown that such a mechanism does indeed play a role in hypoxia-induced changes in intercapillary distances. Such increases in the density of perfused capillaries due to fiber elongation during asphyxia were certainly a determining factor in studies showing densities higher than those observed during normoxia.4

In studies by Honig and coworkers,1,3,4 it was also observed that decreases in intercapillary distances resulted when the arterial PO2 decreased to 30–40 mm Hg due to respiration of hypoxic gas mixtures. These results appear to contradict the view that fiber elongation is involved in such cases. In the present investigation, when the oxygen content in breathing air was reduced to an arterial PO2 of 35 mm Hg, no changes in density of perfused capillaries, in fibers, or in segment lengths were found. However, in contrast to the present study, the measurement of intercapillary distances was not performed during steady-state conditions but was obtained during the initial phase of breathing a 5% O2 gas mixture. Such a concentration cannot be tolerated in a steady state and induces progressive decrease in arterial PO2. This point appears rather important since changes in fiber dilation have been observed to depend not only on the absolute arterial PO2 attained but also on the speed at which hypoxia occurs. With graded reduction in respiratory O2 content, dilation was not observed until the arterial PO2 had attained a value of somewhat less than 20 mm Hg within 3 minutes. In contrast, fiber dilation was highly significant when an arterial PO2 of 35 mm Hg was attained after 30 seconds of asphyxia, an effect that begins instantaneously with interruption of ventilation (Figure 2). The most evident difference between these conditions is related to the time factor involved in the development of hypoxia. In contrast to the sudden heart hypoxia brought about by asphyxia, reduced O2 in breathing air still allows compensatory mechanisms to develop. For example, the blood pressure, a parameter reflecting sympathetic activity, was found to return to normotensive levels especially in those groups subjected to very low oxygen content in the breathing air. It is known from in vitro experiments that hypoxia leads directly to a concentration-dependent decrease in contractility26 but that in vivo sympathetic stimulation, greatly varying in intensity with time, is superimposed on these direct depressant effects.27 Thus, the present observations may be interpreted as follows: Graded increases in hypoxic stress led to a compensation in cardiac contractility due to sympathetic stimulation, whereas hypoxia caused by asphyxia set in so rapidly that cerebral anoxia became manifest before compensatory increases in sympathetic activity could arise.
Considering the results obtained by Honig's group,\textsuperscript{1-3,4} it appears that the effect of breathing a 5% O\textsubscript{2} gas mixture for a short period is more similar to ventilation with 8% O\textsubscript{2} or respiratory arrest than to conditions leading gradually to an identical P\textsubscript{O\textsubscript{2}}. Since both measures (8% O\textsubscript{2} and asphyxia) led to fiber elongation, it appears highly likely that ventilation with 5% O\textsubscript{2} also induces such an effect.

Despite these changes a second possible mechanism might be important for the hypoxia-induced increases in functional capillary density. Changes in the distribution of red cells within the capillary system might play a role here. Increases in intercapillary distances have been observed by Honig and coworkers by counting capillaries containing red cells. It has been postulated that the increase in functional density is due to an increase in capillaries perfused with erythrocytes.\textsuperscript{19} The present experiments concerned with red cell detection in histological sections have been performed with this concept in mind. However, no such changes were demonstrated, at least with respect to the fraction of plasma gaps in capillaries. There was nothing that offers proof of a correlation between the number of capillaries devoid of red cells and the degree of hypoxia.

In this comparison it should be noted, however, that the filming of myocardial capillaries by Honig and coworkers\textsuperscript{1-3,4} was done during the earliest phase of hypoxia before the arterial blood pressure began to fall drastically. Different values of local hematocrits may have been present during this period and might differ from the data obtained during steady-state conditions when the arterial blood pressure has already declined to a rather low level.

Although the latter considerations appear compatible with the conception that in the rat heart perfusion takes place in all capillaries within a few seconds, other studies contradict this view. By again labeling the plasma with a fluorescent dye, Weiss and Conway\textsuperscript{13} reported on a twofold functional reserve in the microvasculature of the heart that could be mobilized by hypoxia. However, these experiments were performed in rabbits, and progressive filling with time was evident. Most of the capillaries were perfused within less than 45 seconds. The longer period required for complete filling in the rabbit can, at least in part, be attributed to the fact that the circulation times in rabbits are greater than in rats. This becomes especially significant when the dye has to travel from the femoral vein, the point of injection, through the vascular system to the coronary ostia.

Capillary recruitment has also been postulated on the basis of tracer distribution analyses.\textsuperscript{8-11,28} Nevertheless, these results do not necessarily contradict those observations reported above if one defines capillary recruitment as the fraction of capillaries perfused at a given point in time. If flow and stagnation frequently change within the microvascular system, rapid shifts in this pattern would change the surface area available for exchange even if the number of capillaries open to flow remains constant.\textsuperscript{29}

The present observations show that real decreases in the distances between such perfused capillaries occur as a result of dilation of the ventricular chambers. This mechanism is especially relevant during extreme hypoxic stress.

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References

17. Fleckenstein A, Döring HJ, Janke J, Byron YK: Basic actions of ions and drugs on myocardial high-energy phosphate metabolism and contractility, in Schmier J, Eichler O (eds): Handbook of Experimental Pharmacology, Volume...


21. Mann HB, Whitney DR: On a test of whether one of two random variables is stochastically larger than the other. Ann Math Statist 1947;18:50-60


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F Vetterlein, H Hemeling, J Sammler, A Pethö and G Schmidt

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