Oxygen distribution in microcirculation after arginine vasopressin-induced arteriolar vasoconstriction


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Friesenecker, B., A. G. Tsai, M. W. Dünser, A. J. Mayr, J. Martini, H. Knotzer, W. Hasibeder, and M. Intaglietta. Oxygen distribution in microcirculation after arginine vasopressin-induced arteriolar vasoconstriction. Am J Physiol Heart Circ Physiol 287: H1792–H1800, 2004. First published 10 June 2004; 10.1152/ajpheart.00283.2004.—The microvascular distribution of oxygen was studied in the arterioles and venules of the awake hamster window chamber preparation to determine the contribution of vascular smooth muscle contraction to oxygen consumption of the microvascular wall during arginine vasopressin (AVP)-induced vasoconstriction. AVP was infused intravenously at the clinical dosage (0.0001 IU·kg⁻¹·min⁻¹) and caused a significant arteriolar constriction, decreased microvascular flow and functional capillary density, and a substantial rise in arteriolar vessel wall transmural PO₂ difference. AVP caused tissue PO₂ to be significantly lowered from 25.4 ± 7.4 to 7.2 ± 5.8 mmHg; however, total oxygen extraction by the microcirculation increased by 25%. The increased extraction, lowered tissue PO₂, and increased wall oxygen concentration gradient are compatible with the hypothesis that vasoconstriction significantly increases vessel wall oxygen consumption, which in this model appears to constitute an important oxygen-consuming compartment. This conclusion was supported by the finding that the small percentage of the vessels that dilated in these experiments had a vessel wall oxygen gradient that was smaller than control and which was not determined by changes in tissue PO₂. These findings show that AVP administration, which reduces oxygen supply by vasoconstriction, may further impair tissue oxygenation by the additional oxygen consumption of the microcirculation.

vasoactivity; oxygen gradients; tissue oxygenation; vasomotion; metabolism

ARGININE VASOPRESSIN (AVP) is a potent endogenous vasopressor hormone and one of the strongest vasoconstrictors in the skin (4–6). This hormone is used clinically to restore blood pressure in severely ill patients, particularly in situations of multiple organ failure and shock, under the assumption that restoration of blood pressure is a key factor in resuscitation, independently of the causative mechanism. However, AVP restores blood pressure via vasoconstriction, an effect that decreases functional capillary density (FCD), a condition shown to be associated with lethality in experimental studies of hemorrhagic shock (13). The clinical use of AVP has also been shown to produce severe ischemia of the skin and limbs (5), raising the question of whether the severity of these reactions may be related to the combined effect of decreased tissue perfusion and oxygen delivery. New information on the distribution of oxygen in the different tissue compartments of the organism (35) identifies the arteriolar vessel wall as an oxygen-consuming compartment whose share of total oxygen availability increases as a consequence of energy consumption by the arteriolar wall to maintain vasoconstriction. Therefore, the potential exists that the administration of AVP may take the organism to an intrinsically unstable condition whereby the benefit derived by increased perfusion pressure, is negated by the synergistic combination of decreased oxygen delivery, FCD, and increased arteriolar wall oxygen consumption.

Analysis of the relative contributions of blood flow and tissue oxygen consumption in determining tissue PO₂ during vasoconstriction requires the measurement of their distribution at the microvascular level. The oxygen distribution in the microcirculation under normal conditions was reviewed by Tsi et al. (35), who analyzed the available information and reached the conclusion that in some tissues, oxygen is supplied prevalently by the arterioles. In these studies, distribution of oxygen was reported as a function of vessel size, a parameter that is not entirely appropriate in situations where vessel size changes due to vasoactivity. A more definitive method for characterizing the distribution of PO₂ along the microvascular network consists of classifying arterioles and venules according their hierarchical order of branching and measuring PO₂ at the beginning of each branching order (35). This procedure is particularly suitable for investigating the tissue of the Syrian Golden hamster window preparation, which presents a consistent pattern of branching, thus allowing different comparison microvascular network responses in the presence of substantial changes of vessel caliber.

Network studies of the microcirculation show a branching order-dependent perivascular PO₂ gradient is present in both arterioles and venules. The discovery of this gradient, rendered possible by the measurement of blood and tissue PO₂ at the interface between blood and tissue (34), resolved, in part, mass balance anomalies discovered in early studies of oxygen distribution in the microcirculation (2, 3, 19). The presence of large arteriolar vessel wall oxygen gradients led to the hypothesis that their magnitude is due to oxygen consumption by the vessel wall, namely the combination of endothelium and...
smooth muscle, which are the primary cellular components of these vessels.

Vascular smooth muscle is the main component of arteriolar vessel walls. Scanning electron microscopic observations of arterioles in the dog brain show a direct correlation between arteriolar vessel size and number of vascular smooth muscle cell layers (27). In contrast, venular vessel walls mainly consist of endothelium surrounded by differently shaped pericytes; only large venules are surrounded by a discontinuous layer of vascular smooth muscle (9). In working skeletal muscle, oxygen uptake and energy turnover are directly related during exercise-induced contractions (8); by contrast, smooth muscle supposedly maintains tone with minimal energy expenditure and presumably oxygen consumption (18). One may conjure a mechanism by which the arterial wall maintains tone with minimal energy consumption; however, such a low-energy consuming process would seem to be unlikely if the vessel caliber changes continuously as the circulation autoregulates on a moment-to-moment basis. Furthermore, increased tone or caliber changes continuously as the circulation autoregulates.

The present study was designed to investigate the following: 1) how oxygen is distributed in the microcirculation of the hamster window chamber after application of AVP in a clinically relevant dosage; and 2) how oxygen is delivered to the tissue under conditions of pharmacologically induced severe vasoconstriction.

MATERIALS AND METHODS

Animal Model and Preparation

The animal experiments were approved by the Austrian Ministry of Science and Research. While the animals were under pentobarbital anesthesia (50 mg/kg body wt), a viewing window was inserted into the dorsal skinfold of 13 male golden Syrian hamsters (weight 60–85 g). The basic technique has been described in detail elsewhere (1, 7, 11). Briefly, the dorsal skinfold consisting of two layers of skin and corresponding muscle tissue was placed between two titanium frames. A 15-mm circular portion of the skin, including two skin muscles, was carefully removed, so that only one thin monolayer of skin muscle with the underlying skin remained in place. The tissue was covered with saline, and a cover glass was held by one side of the titanium head to allow subcutaneous to the base of the chamber, exteriorized through the skin as it equilibrates through the tissue fluid after exiting the more permeable capillaries and venules. Interstitial PO2 measurements are feasible although the arteriolar wall has a very low permeability to albumin because the dye reaches this portion of the tissue after partitioning into the interstitial space from the more permeable vessels in the tissue.

Inclusion criteria. Animals were suitable for the experiments if 1) systemic parameters were within normal range, namely, heart rate (HR) >340 beats/min, mean arterial pressure (MAP) >80 mmHg, systemic hematocrit (Hct) >45%; and arterial PO2 >50 mmHg and 2) microscopic examination of the tissue in the chamber observed under ×600 magnification did not reveal signs of edema or bleeding.

Systemic Parameters

MAP was tracked periodically during the experiment via the arterial catheter, and HR was determined from the pressure trace (Recom pressure transducer system, model 13-6615-50; Gould Instrument Systems). Systemic Hct was measured from centrifuged arterial blood samples taken in heparinized capillary tubes (Hettich Hct 24 centrifuge, Tuttingen, Germany).

Blood Chemistry

Arterial blood was sampled from the carotid artery catheter into a heparinized capillary tube and immediately analyzed for PO2, PCO2, and pH at 37°C (Blood Chemistry Analyzer 248, Ciba-Corning; Essex, UK). The blood and plasma hemoglobin (Hb) content was determined with the use a hand-held photometer (B-Hemoglobin Photometer; Hemocue) from a drop of blood.

Functional Capillary Density

Detailed mappings were made of the chamber vasculature so that the same vessels were studied throughout the experiment. Capillary segments were considered functional if red blood cells (RBCs) were observed to transit through the capillary segments during a 45-s period. FCD is tabulated from the capillary lengths with RBC perfusion (10) divided by the area of the microscopic field of view.

Microhemodynamic Parameters

Arteriolar and venular blood flow velocity are measured online using the photodiode cross-correlation technique. (Fiber Optic Photo Diode Pickup and Velocity Tracker model 102B-C; Vista Electronics, San Diego, CA). The video image shearing technique is used to measure vessel diameter (D) on-line (model 908; Vista Electronics, San Diego, CA). The measured centerline velocity (V) was corrected according to vessel size to obtain the mean RBC velocity (14). Blood flow was calculated from the measured parameters as Q = V·π(D/2)2.

Microvascular PO2 Distribution

High-resolution microvascular PO2 measurements were made using phosphorescence quenching microscopy (31). This noninvasive method of measuring oxygen levels is based on the oxygen-dependent quenching of phosphorescence emitted by albumin-bound metallophyrin complex after pulsed light excitation. With the use of this technique, PO2 measurements are obtained by determining the rate of decay of the excited phosphorescence, which is inversely proportional to the amount of oxygen that surrounds the dye and independent of the amount dye present or the intensity of light excitation provided that the signal-to-noise ratio is adequate. The phosphorescence decay curves were converted to PO2 values with the use of a fluorescence decay curve fitter (model 802, Vista Electronics; Ramona, CA) (12). This technique has been used in this animal preparation and others for both intravascular and extravascular PO2 measurements because albumin exchange between plasma and tissue allows for sufficient concentrations of albumin-bound dye within the interstitium to achieve an adequate signal-to-noise ratio. Measurements of PO2 in the periarteriolar tissue are feasible although the arteriolar wall has a very low permeability to albumin because the dye reaches this portion of the tissue as it equilibrates through the tissue fluid after exiting the more permeable capillaries and venules. Interstitial PO2 measurements made with this system have been compared with simultaneous measurements with recessed oxygen electrodes and their differences were found not to be statistically significant (34). Animals received a slow intravenous injection of 15 mg/kg body wt at a concentration of 10.1 mg/ml of a palladium-meso-tetra(4-carboxyphenyl) porphyrin (Porphyrin Products; Logan, UT). The dye is allowed to circulate for 20 min before PO2 measurements at which point enough dye has entered into the interstitial space from the more permeable vessels in the network.

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In our system, intravascular measurements are made by placing an optical rectangular window within the vessel of interest and the longest side of the rectangle is positioned parallel to the vessel wall. Measurements are made by optically placing a rectangular window region of $\sim 5 \times 40 \, \mu m$ longitudinally within the vessel. Tissue $P_O_2$ was measured in regions void of large vessels within intercapillary spaces with an optical window size of $\sim 10 \times 10 \, \mu m$. Thus, exact localization of the $P_O_2$ measurements is known, whether intravascular in an arteriole or a venule or in the interstitial tissue. Close localization is not possible with needle or surface array electrode techniques. Results from this type of measurement allow for detailed understanding of microvascular oxygen delivery and most importantly to assess oxygen uptake by the tissue. The phosphorescence decay due to quenching at a specific $P_O_2$ yields a single decay constant and in vitro calibration has been demonstrated to be valid for in vivo measurements. The use of this technique with the window chamber model assures a high-resolution optical noninvasive method of assessing microvascular oxygen distribution in an intact tissue.

Intravascular and perivascular $P_O_2$ measurements were made immediately after branching bifurcation points. Intersitial tissue $P_O_2$ was measured within the interstitium far away from visible underlying and adjacent vessels.

**Global Oxygen Transport Parameters**

Calculations of oxygen transport at the microvascular level are determined from the microvascular measurements. Oxygen release by the blood is the difference between the oxygen content at the arterial ($A$) and venular ($V$) segments of the microvascular network times the average flow through the tissue and expressed as

$$O_2 \text{ release by blood} = (RBC_{Hb} \times \gamma \times S_A \%) + [(1 - Hct) \times \alpha \times P_{O_2 A-V}] \times Q_M$$

where $RBC_{Hb}$ is the hemoglobin concentration in red blood cells (g/dl blood), $\gamma$ is the oxygen-carrying capacity of hemoglobin at 100% saturation or 1.34 ml O$_2$/g Hb, $S_A \%$ is the arteriolar-venous difference in RBC $O_2$ saturation, $(1 - Hct)$ is the fractional plasma volume and converts the equation from per deciliter of plasma to per deciliter of blood, $\alpha$ is the solubility of oxygen in plasma, $3.14 \times 10^{-3}$ ml O$_2$/dl plasma mmHg, and $Q_M$ is the volumetric blood flow calculated as the averaged microvascular flow. The hemoglobin saturation is determined from the $P_O_2$ using the oxygen dissociation curve for hamster blood (36).

**Experimental Setup**

The unanesthetized animal was placed in a restraining tube that was stabilized by affixing the tube and the chamber to a Plexiglas plate. The animal had free access to wet feed during the entire experimental period. The Plexiglas stage that held the animal was then placed on an intravitral microscope (Mikron Instruments; San Diego, CA) equipped with two lamp sources (F0–150 halogen fiberoptic illuminator or mercury arc lamp Gemini 300 fiberoptic high-intensity short arc light source; CHIU Technical; Kings Park, NY) and three infinity-corrected objectives (Zeiss Achromat $\times 20/0.50$ W, $\times 40/0.75$ W, and $\times 65/0.90$ W). The tissue image was projected onto a charge-coupled device camera (model COHU FK 6990 IQ-S, Pieper; Düsseldorf, Germany) and viewed on a monitor (model PVM-1454QM; Sony). The animal was allowed a 30-min adjustment period to the tube environment before the baseline systemic parameters (MAP, HR, arteriolar blood gases, and Hct) were measured. Microvascular fields of study were chosen by their visual clarity. A 420-nm blue filter was used for contrast enhancement of the transillumination image. Functional capillary density was assessed. Arterioles and venules (4–6 of each type) were characterized by their diameter and blood flow velocity.

**Experimental Design and Drug Dosage**

Preliminary dose response experiments showed that adequate microcirculatory flow in the presence of well-defined arteriolar vasoconstriction could be obtained with the clinically used AVP dosage of 0.0001 IU/kg·min$^{-1}$. Increasing the dosage further resulted in extreme constriction and occasional flow cessation in A0 vessels and a no/low flow condition within the chamber network.

Studies were performed in two groups of animals randomly assigned to either the control or the AVP-treated group. Baseline systemic and microhemodynamic characterizations were performed in both groups of animals. The treatment group was then subjected to a continuous intravenous infusion of AVP at a rate of 0.0001 IU·kg$^{-1}$·min$^{-1}$, which was found in pilot studies to produce a consistent and stable level of vasoconstriction without completely eliminating blood flow, which occurred at higher dosages. After 30 min of treatment, microhemodynamic and $P_O_2$ distribution attained a steady state and measurements were performed. In the control group, $P_O_2$ distribution measurements were performed with the microhemodynamic measurements at baseline.

**Statistical Analysis**

All measurements were compared with corresponding values at baseline prior to treatment with AVP except for the microvascular oxygen measurements in which the $P_O_2$ levels were compared between the two groups of animals: control and AVP treated. Comparisons were made with the paired $t$-test (Prism 4.0, GraphPad; San Diego, CA). Changes are considered statistically significant if $P < 0.05$. Data is presented as means ± SD unless otherwise noted. Trends, independent of the scatter, are shown as means ± SE values. Data are presented as absolute values and ratios relative to baseline values. A ratio of 1.0 signifies no change from baseline, whereas lower or higher numbers are indicative of higher or lower values than baseline. Calculations of global oxygen transport parameters that are not directly measurable in our model are based on mean values of measured parameters. These calculations are presented without standard deviations to focus on trends rather than the variability of measurements.

**RESULTS**

Thirteen animals that followed all inclusion criteria were entered into the study and randomly assigned to the two experimental groups: control ($n = 7$) and AVP ($n = 6$). All animals completed the protocols without any visible signs of discomfort. Animals were observed resting and periodically eating moist feed throughout the experimental period. No statistical differences were found in one-way ANOVA of the systemic and microvascular data at baseline in both experimental groups.

**Systemic Parameters**

AVP treatment caused an increase in MAP by $1.37 \pm 0.09$ (91.3 ± 6.4 to 124.7 ± 4.4 mmHg) and decrease in HR by $0.88 \pm 0.05$ (447.5 ± 44.9 to 395.0 ± 52.5 beats/min) of baseline ($P < 0.05$). There were no statistically significant changes to the blood gas parameters as a result of AVP treatment.

**Microvascular Hemodynamics**

The changes in vessel diameter in response to AVP treatment over the entire microvascular network are shown in Fig. 1. Most arteriolar levels of branching constricted; the reduced diameters were significantly smaller than baseline: A0 ($0.61 \pm$
The RBC velocity was statistically reduced in all branches of the vascular network. *P < 0.05. Relative to baseline, the arterioles (A0, A1, A3, and A4) tended to vasoconstrict, whereas the A2 vessels were unchanged from baseline. The venules, large venules (VL) and collecting venules (VC) vasodilated, whereas the largest draining venule slightly vasoconstricted from baseline. The dotted line represents the baseline level, therefore values above and below the line are indicative of vasodilation and vasoconstriction, respectively. *P < 0.05, except in the VC vessels where large increases in flow were also observed.

Table 1. Microhemodynamic changes in microvascular network in response to AVP infusion

<table>
<thead>
<tr>
<th>Baseline Diameter, μm</th>
<th>AVP Treatment, %</th>
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<tbody>
<tr>
<td>Diameter</td>
<td>Constrict VM Flow</td>
</tr>
<tr>
<td>A0 129.0 ± 7.3</td>
<td>51.2 ± 7.1*</td>
</tr>
<tr>
<td>A1 47.2 ± 13.5</td>
<td>76.5 ± 23.0*</td>
</tr>
<tr>
<td>A2 23.7 ± 7.5</td>
<td>89.5 ± 44.6</td>
</tr>
<tr>
<td>A3 14.9 ± 5.5</td>
<td>76.6 ± 41.2*</td>
</tr>
<tr>
<td>A4 9.1 ± 2.4</td>
<td>80.5 ± 21.2*</td>
</tr>
<tr>
<td>VC 34.7 ± 7.5</td>
<td>133.2 ± 26.9*</td>
</tr>
<tr>
<td>VL 97.8 ± 39.9</td>
<td>117.1 ± 14.8*</td>
</tr>
<tr>
<td>V0 395 ± 92.4</td>
<td>95.0 ± 1.5*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of vessels studied. AVP, arginine vasopressin; VM, vasoconstriction; A0–A4, arterioles; VC, collecting venules; VL, large venules; V0, baseline draining vein. Changes in diameter and flow are shown at baseline before and during AVP treatment. Diameter (% relative to baseline); %constrict, percentage of vessels that constricted; %VM, percentage of vessels that constricted and had vasomotion, flow (% relative to baseline) *P < 0.05 relative to baseline.

Table 1 summarizes: 1) vessel sizes at baseline for the different vessel orders in the network; 2) diameter and flow changes relative to baseline; and, 3) the percentage of vessels constricting and exhibiting vasomotion during AVP treatment.

Figure 3 is a low-magnification photo taken with a ×10 objective of a microvascular field to illustrate the typical changes to the vessel diameters in response to AVP treatment. The normal baseline is shown in the right panel and after AVP treatment arterioles are constricted and venules dilated.

**Functional Capillary Density**

FCD was reduced to 0.62 ± 0.70 of baseline during AVP infusion (P < 0.05).

**Oxygen Distribution in the Microvascular Network**

Figure 4 shows the intravascular and perivascular P O 2 levels in the microvascular network obtained in the control (Fig. 4, A and C) and AVP-treated groups (Fig. 4, B and D). Intravascular P O 2 and transmural vessel wall P O 2 gradient for all segments of the microvascular network are presented in Table 2.

**Arterioles.** A vessel size-dependent drop in longitudinal intravascular P O 2 was observed in both control and AVP groups. Intravascular P O 2 tended to be slightly lower in A0 vessels during AVP infusion (P < 0.05), but was not different in A1–A4 arterioles compared with the control group. Transmural vessel wall P O 2 gradient at all levels of the vasculature, calculated from the difference between the intravascular P O 2 and perivascular P O 2 was increased due to the significantly reduced perivascular P O 2 during AVP infusion (P < 0.05).

**Venules.** Venular intravascular and perivascular P O 2 were significantly lowered during AVP infusion in each vessel category. During AVP infusion venular transmural vessel wall P O 2 gradient was unchanged in V0 and decreased in dilating VC and VL vessels compared with the control group (P < 0.05).

**Tissue P O 2.** Tissue P O 2 was significantly reduced to 7.2 ± 5.8 mmHg during AVP infusion compared with the control level of 25.4 ± 7.4 mmHg (P < 0.05).
Microvascular Flow and Microvascular Oxygen Delivery

Table 1 shows the changes of diameter and flow in the microvascular network in response to AVP administration shown relative to baseline after AVP vasopressin administration. The overall change in microvascular flow through the network was determined by averaging microvascular flow for all the microvessels because of the heterogenous responses in the network. This lack of uniformity is due to the presence of interconnecting (arcading) arterioles within a given vessel order. The averaged flow has been found to closely correspond to the change in cardiac index in previous studies (33). Oxygen release in the control and the AVP-treated group was calculated using the intravascular PO2 in A0 and V0 vessels (Table 2) and the average flow data. Oxygen saturation in A0 and V0 was 91% and 63% for control, and 89% and 31% for AVP respectively. The data of Table 1 shows that the average flow due to AVP vasoconstriction decreased to 0.61 of baseline. Therefore, oxygen release in the control group was \( \frac{0.91 - 0.63}{0.63} \times 1.000 = 0.28 \) (ml O2/unit time, arbitrary units) and in the AVP-treated group was \( \frac{0.89 - 0.31}{0.61} = 0.35 \) (ml O2/unit time, arbitrary units referred to control), an increase of 25% in the treated group.

DISCUSSION

The principal finding of this study is that the administration of a continuous intravenous AVP infusion applied at a clinically relevant dosage decreased the rate of oxygen delivery by the arteriolar vessels, increased the rate of oxygen release to the microcirculation and reduced tissue PO2. Administration of AVP reduced arteriolar diameter and microvascular flow, significantly decreased functional capillary density and substantially raised arteriolar vessel wall transmural PO2. These phenomena were accompanied by the onset of vasomotion, which was most pronounced in smaller arterioles (A2–A4) and the increase of oxygen consumption by the tissue during AVP treatment, relative to control. Arteriolar vasomotion is a mechanism present during pathological perfusion and mostly absent during normal baseline conditions (23, 26). The appearance of vasomotion after the administration of AVP is indicative of evolving tissue hypoxia in the tissue of the awake hamster.

![Baseline and AVP Treatment](image_url)

Fig. 3. Photomicrographs taken at low magnification showing the typical microvascular response to AVP treatment. The left and right photos are taken at baseline and after AVP treatment, respectively. The arteriole marked at sites 1–3 in the left photo constricted and their diameters were 55, 54, and 61% of baseline, respectively, after treatment (right). The collecting venule denoted with 4 and the large venule (site 5 and 6) dilated by 13, 9, and 7%, respectively.

![Intravascular and Perivascular PO2 Distribution](image_url)

Fig. 4. Intravascular and perivascular PO2 distribution in the microvascular network and tissue in control and after AVP treatment. The arteriolar intravascular PO2 was no different between the two groups; however, tissue and venular intravascular PO2 was significantly reduced. Perivascular PO2 was significantly reduced in all levels of the vasculature. *P < 0.05 relative to the control group.
which governs the exchange of oxygen between the microvessels and the tissue. The normal oxygen tension (PO2) at the outer wall of the microvessels is in equilibrium with the tissue. A4 arterioles, which are the larger vessels, supply PO2 to the tissue. The arteriolar microcirculation in the control and AVP-treated groups was calculated using the difference in PO2 between the arterioles and the tissue; therefore, the oxygen source for this increased PO2 should be related to the various forms of direct or indirect oxygen shunting from large arterioles to the tissue. In terms of how oxygen is distributed in control/normal conditions in the tissue under study, this analysis shows a greater role being played by the arteriolar oxygen consumption than that previously reported. The principal difference between these two studies is that in the present study is systematized specifically to branching order, whereas the previous study, the data was analyzed as a function of vessel size.

### Table 2. Oxygen distribution in microvascular network

<table>
<thead>
<tr>
<th>Microvascular PO2 Distribution During AVP Treatment</th>
<th>Control, mmHg</th>
<th>AVP Treatment, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>iP O2</td>
<td>O2 gradient</td>
<td>iP O2</td>
</tr>
<tr>
<td>A0</td>
<td>67.5 ± 2.2 (9)</td>
<td>18.7 ± 4.4</td>
</tr>
<tr>
<td>A1</td>
<td>56.4 ± 6.4 (35)</td>
<td>20.1 ± 5.0</td>
</tr>
<tr>
<td>A2</td>
<td>49.1 ± 7.7 (19)</td>
<td>19.9 ± 4.3</td>
</tr>
<tr>
<td>A3</td>
<td>39.3 ± 7.5 (22)</td>
<td>16.9 ± 4.0</td>
</tr>
<tr>
<td>A4</td>
<td>27.2 ± 6.0 (24)</td>
<td>11.9 ± 3.5</td>
</tr>
<tr>
<td>Tissue</td>
<td>25.4 ± 7.4 (84)</td>
<td>7.2 ± 5.8 (84)*</td>
</tr>
<tr>
<td>VC</td>
<td>21.6 ± 3.7 (49)</td>
<td>8.6 ± 1.9</td>
</tr>
<tr>
<td>VL</td>
<td>28.3 ± 5.5 (44)</td>
<td>11.9 ± 3.8</td>
</tr>
<tr>
<td>Vo</td>
<td>39.8 ± 3.7 (9)</td>
<td>18.0 ± 2.9</td>
</tr>
</tbody>
</table>

Values are means ± SD; numbers in parentheses refer to the number of measurements of the vessels. iP O2, intravascular PO2; pPO2, perivascular PO2; iP O2 in each network segment and in interstitial tissue region is shown. O2 gradient calculated from the difference between iP O2 and pPO2. *P < 0.05, statistical significance between AVP and control groups.

Venular Dilation during AVP Infusion

Venular dilation during continuous AVP infusion was most pronounced in the smaller collecting vessels. Significant arteriolar vasoconstriction and, hence, drastic reduction of arteriolar blood flow usually causes simultaneous venular constriction because of a significant decrease in transvenular distending pressure, therefore this finding is unusual (22), also considering that Marshall showed that vasopressin released during systemic hypoxia exerts a constrictor influence on proximal arterioles and all sections of the venous tree of spinotrapezius muscle in rats (15). However, mechanisms for the active, endothelium-dependent relaxation of veins, such as postjunctional β-receptor activation (38), or bradykinin-induced endothelial cell activation (10) have been described. Because the venular endothelium has a large variety of receptors that mediate both venular constriction and venular dilation (α1, α2, β2, dopaminergic, muscarinic, angiotensin, bradykinin, vasopressin, D-tryptamine, H1, H2, and prostaglandin receptors) (37) there is a possibility that this phenomenon resulting from a balance between different receptor responses is species dependent.

### P O2 Distribution in Microvasculature and Oxygen Release in Control

The intravascular distribution of PO2 in the control group (Fig. 4) shows that in this tissue under normal conditions most of the oxygen is contributed by the arterioles because the PO2 of A4-VC vessels is in equilibrium with the tissue. However, the actual source of oxygen to the tissue is not blood but the blood/blood vessel combination taken as a unit. Therefore, the PO2 that determines the rate of exchange of oxygen from the blood vessel to the tissue is the PO2; at the outer microvessel surface, which is obtained by subtracting the vessel wall oxygen gradient from the intraluminally measured PO2. Table 2 also shows the oxygen distribution in the tissue and the difference in PO2 (ΔPO2) measured across the vessel wall, and Figs. 4C shows the normal PO2 of the outer microvessel wall, which is the PO2 of the oxygen source “seen” by the tissue, which governs the exchange of oxygen between the microblood vessels and tissue. Tissue PO2 and outer vessel wall of A3 arterioles are the same. Furthermore, tissue PO2 is higher than PO2 at the outer wall of A4 arterioles. Considering that oxygen diffuses in the direction of the negative oxygen concentration gradient it appears that: 1) oxygen to the tissue is supplied by the A0 and A1 arterioles; 2) there is net oxygen flux between A2 and A3 arterioles and tissue; 3) there is a net flux of oxygen from the tissue to the outer wall of A4 arterioles; and 4) a portion of the oxygen delivered from the arterioles to the tissue is shunted to the venular system.

Data from Tables 1 and 2 allow calculation of the rate of oxygen delivery by various segments of the arteriolar microcirculation by noting the difference in blood oxygen saturation between microvessels and multiplying this difference by the relative flows. It is of interest that in control condition, oxygen released from the A3 to the VC vessels cannot reach the tissue, because tissue PO2 is higher than the PO2 of the outer wall of these vessels; therefore, presumably this oxygen is either consumed by the arteriolar vessel wall or shunted to the venous exit.

In analyzing the balance of oxygen delivery in the microcirculation it is also important to consider that because the outer surface of V0 venules PO2 is in equilibrium with tissue PO2, but not with VC and VL venules, there may be some transferral of oxygen from the tissue to the venules. However, intraluminal PO2 in V0 venules is statistically higher than tissue PO2; therefore, the oxygen source for this increased PO2 should be related to the various forms of direct or indirect oxygen shunting from large arterioles to the tissue to the venules.

In terms of how oxygen is distributed in control/normal conditions in the tissue under study, this analysis shows a much greater role being played by the arteriolar oxygen consumption than that previously reported (11). The principal difference between these two studies is the method for assigning PO2 values to a given vessel branch, which in the present study is systematized specifically to branching order, whereas in the previous study, the data was analyzed as a function of vessel size.

### P O2 Distribution in the Microvasculature and Oxygen Delivery During AVP-Induced Vasocostriction

Administration of AVP does not significantly change the intravascular arteriolar PO2 distribution pattern (Fig. 4B) which is essentially identical to that found in normal/control conditions. However, venular PO2 values are significantly lower, which is indicative of greater oxygen extraction during AVP infusion, because in the venular range of PO2, the oxygen dissociation curve for hemoglobin is steepest. Vasocostriction significantly affects the global rate of oxygen release by the arteriolar microcirculation calculated using the difference in blood oxygen saturation between A0 and VC vessels multiplied by blood flow, which lowered to 0.73 of control from the nominal value of [0.91 ± 0.20] × 1.000 = 0.71 (ml O2/unit time, arbitrary units) to [0.89 ± 0.03] × 0.611 = 0.52 (ml O2/unit time, arbitrary units referred to control) in the AVP-treated group. However, the tissue as a whole receives more oxygen because, as indicated in RESULTS, the net oxygen released to the tissue is increased by 25%. This apparent paradox is due to the higher oxygen content in the venular circulation where V0 blood is 63% saturated in control conditions versus...
30% saturated during AVP treatment. Therefore, under control conditions there is a greater amount of oxygen that is shunted to the venous return, whereas during AVP treatment although globally less oxygen is convectively brought to the tissue, more oxygen is extracted presumably due to increased oxygen consumption.

To reconcile the disparities in oxygen release and consumption between control and AVP treatment we note that the oxygen released from the arterioles during AVP treatment does not reach the tissue, as indicated by the large vessel wall oxygen gradients. As a consequence the outer wall of the arteriolar blood vessels has a low $P_{O2}$, and tissue $P_{O2}$ is correspondingly low, two factors that combine in lowering the gradient driving oxygen into the venular circulation, which lowers oxygen shunting into the venular return and increases extraction. These factors suggest the following scenario for AVP-induced vasoconstriction. First, increase of the arteriolar vessel oxygen gradient due to mechanisms related to increased vessel wall oxygen consumption. Second, lowered tissue $P_{O2}$ due to lowered oxygen availability to the tissue resulting from decreased blood flow and increased vessel wall oxygen consumption. Third, compensatory increase in global oxygen delivery by the microcirculation due to decreased arteriolar/venular oxygen shunting and therefore increased extraction.

Although the relative contribution of each of the changes produced by AVP treatment cannot be accurately assessed, the fact remains that increased oxygen release into the microcirculation results in a significantly decreased tissue $P_{O2}$ and FCD. This finding indicates that the extra oxygen delivered is being consumed by a tissue compartment that becomes active during vasoconstriction. Because the tissue under study is at rest in control and during AVP treatment, the remaining tissue likely to expend energy is the microvasculature, requiring the expenditure of energy to maintain time-dependent tone.

Oxygen release by the capillaries during AVP treatment is higher than in the control group even though FCD and overall flow in the microcirculation was lower. This supports the concept that tissue $P_{O2}$ is governed by the oxygen supply from both capillaries and arterioles. Increasing the oxygen supply from capillaries does not necessarily lead to an increased (or maintained) tissue $P_{O2}$ if the oxygen supply from arterioles is significantly lowered due to increased arteriolar wall oxygen consumption, as shown by the increased wall $P_{O2}$ gradient. In this situation tissue $P_{O2}$ may fall even though capillary oxygen delivery is increased as shown in this study. Therefore in these experiments the reduction of FCD does not explain the reduction of tissue $P_{O2}$ because fewer capillaries (relative to control) deliver more oxygen (than in control).

**Oxygen Consumption by the Microvascular Wall**

The rate of vessel wall oxygen consumption per unit volume of tissue, $g_w$, is directly proportional to $\Delta P_{O2}$ measured across the vessel wall found in these experiments according to the relationship derived by Tsai et al. (32)

$$ g_w = \alpha D \frac{\Delta P_{O2}}{w \delta} \quad (2) $$

where $D$ is the diffusion constant for oxygen in the vessel wall, $\alpha$ is the solubility of oxygen in the vessel wall, $w$ is the vessel wall thickness, and $\delta$ is the “penetration depth,” which defines the distance from the blood tissue interface at which the oxygen flux from the blood column is zero. This equation is a simplified approximation of the actual process because it is a solution valid for Cartesian coordinates rather than cylindrical geometry, and is therefore, a better approximation for the larger vessels. However, the direct dependence between oxygen gradient and oxygen consumption is not affected by this approximation, thus our data shows that oxygen consumption by the arteriolar microvasculature, which appears to be a significant portion of the oxygen partition for the tissue studied, increases significantly due to AVP infusion.

The experimental evidence on vessel wall oxygen consumption is based on the measurement of the $\Delta P_{O2}$ between the blood side of the blood/tissue interface, and the tissue side of the same interface. These measurements of the vessel wall $\Delta P_{O2}$ are made systematically placing the measurement window at the point at which the investigator identifies the boundary of the outer vessel wall, leading to a measurement of the vessel wall $\Delta P_{O2}$ (across its thickness) but not a measurement of the vessel wall thickness. The same procedure is used for measurements in control and vasoconstriction; therefore, there is some uncertainty arising from the inability to precisely locate the outer vessel wall boundary. However, the error incurred should be limited because $P_{O2}$ falls steeply within the vessel wall as shown by Tsai et al. (34), and has a significantly shallower decay in the surrounding tissue, thus it is likely that the vessel wall oxygen gradient is underestimated rather than over estimated.

$P_{O2}$ measurements are made so that the phosphorescence emission passes through a narrow window placed parallel the axis of the vessel. This window is imaged via a $\times 40$ 0.75 numerical aperture objective with a depth of focus of $\sim 5 \mu m$, thus there is some integration of phosphorescence emission from both underlying and overlying tissue layers. Because the vessels are embedded in a tissue that is $\sim 200 \mu m$ thick, the oxygen gradient measured in the larger diameter vessels (on the order of 100 $\mu m$) is mostly representative of that present in the lateral direction; however, the exit from the smaller arterioles approximates progressively that of a cylindrical source in an infinite medium as the vessels become smaller. This effect causes an overestimate of the vessel wall $\Delta P_{O2}$ as the vessel sizes decrease. This error, however, should be negligible for the A0 and A1 arterioles.

The finding that AVP-induced vasoconstriction increases oxygen consumption by the vasculature is in agreement with the report of Ye et al. (40). These authors perfused the rat hind limb, kidney, intestine, and the mesentery at constant flow, and found that vasoconstriction induced by the administration of norepinephrine or vasopressin significantly increased the rate of oxygen extraction from the perfusion solution. This study indicated that perfused blood vessels in the mesentery conserved oxygen at the rate of 115 $\mu mol/l h \ g$ wet weight of blood vessels at 25°C and that this value increased by 75% on constriction. Because vasoconstriction lowers functional capillary density (32) and tissue perfusion, as seen in our study, it is unlikely that the increased oxygen consumption was due to a better perfusion of the tissue, although the authors favored that interpretation in later studies (29, 30).

Oxygen consumption is in most instances directly related to mitochondrial activity; however, mitochondrial density is not particularly pronounced within the endothelium (16) and can-
not solely account for the high respiratory rate of the vessel wall. Smooth muscle, which is continuously activated in adjusting vessel diameter on a moment-to-moment basis in response to neurogenic, metabolic, and mechanical stimuli, may also contribute significantly to the overall oxygen consumption of the microvascular wall. The studies of Waypa et al. (39) in the isolated heart and lung preparations also show that mitochondria may be activated by hypoxia, and that oxygen consumption by the hypoxic tissue may be increased by a related production of reactive oxygen species (ROS) acting as a second messenger in response to hypoxia sensing by mitochondria. The studies of Steiner et al. (28) show that ROS formation by hypoxia occurs in the mesenteric microvasculature. In our experiments, neither the vascular wall nor the tissue reached \( \text{PO}_2 \) levels presumed to trigger these responses to hypoxia; however, this potential cascade of reactions may constitute an additional limiting factor to be considered in the administration of AVP to restore blood pressure in severely ill patients with the objective of improving tissue oxygenation.

**Vasomotion and Vessel Wall Oxygen Consumption**

Vasomotion was present in the majority of arterioles observed during AVP treatment as previously reported (17, 20, 25). In the present study, the vessel wall oxygen gradient of A1 arterioles was measured in vessels with and without vasomotion during AVP treatment. We found that the average \( \text{PO}_2 \) wall gradient in static vasoconstricted, and vasomoting A1 arterioles was the same and there was no correlation between vessel wall \( \text{PO}_2 \) gradient and vasomotion activity. These data suggest that rhythmic changes of vascular smooth muscle contraction and relaxation do not increase overall arteriolar oxygen consumption beyond that due to vasoconstriction.

**Vasodilatation and Vessel Wall Oxygen Consumption**

A minor portion of the arterioles vasodilated during continuous AVP infusion. An imbalance of the physiological regulation and inhomogeneous venular flow might have contributed to individual arteriolar dilation in a generally constricted vascular bed of an oxygen-deprived tissue. Significantly increased flow in dilated arterioles might serve as last means to improve and maybe preserve perfusion of tissue at the verge of hypoxia. As opposed to considerably increased transmural vessel wall \( \Delta \text{PO}_2 \) in constricted and vasomoting arterioles, vessel wall \( \text{PO}_2 \) transmural differences of dilated arterioles were the same or slightly below normal, suggesting that the relaxation of vascular smooth muscle is most likely a passive process that does not consume additional oxygen.

The finding that arterioles that vasodilated during AVP treatment lower their wall oxygen gradient further supports the hypothesis that the gradient is related to the changes in vessel wall oxygen consumption and not to the changes in tissue \( \text{PO}_2 \) due to the change in convective oxygen delivery. It could be argued that during vasoconstriction, tissue \( \text{PO}_2 \) lowers because of the reduced oxygen supply, thus increasing the difference between intravascular and tissue \( \text{PO}_2 \) and therefore the wall gradient. However, this difference appears only in the immediate vicinity of the microvessels. Furthermore, arterioles that dilated during AVP treatment were surrounded by a tissue perfused as a whole at a lower blood oxygen convective rate that had an intrinsically lower tissue \( \text{PO}_2 \), thus the wall gradient of these vessels are not a consequence of their oxygen delivery capacity, but appears to be a direct consequence of their tone. The finding that dilating venules uniformly reduced their vessel wall gradients further supports these conclusions.

In conclusion, the present study shows that oxygen delivery to the tissue of hamster window model is primarily determined by the oxygen exit from arterioles, and that this process becomes severely limited as a consequence of AVP treatment, although oxygen delivery by the microcirculation to this tissue is increased. The reduction in oxygen supply due to vasoconstriction is accompanied by the increase in arteriolar wall transmural \( \Delta \text{PO}_2 \), a parameter that is directly related to vessel wall oxygen consumption. The critical finding is that AVP increases the rate of oxygen release in the microcirculation through the increase of oxygen extraction, whereas tissue \( \text{PO}_2 \) is decreased. This finding suggests that vasoconstriction increases oxygen consumption in the vascular compartment to the detriment of oxygen delivery to the tissue. AVP is usually administered to raise blood pressure for the purpose of ensuring tissue perfusion; however, this effect is achieved via vasoconstriction, which is shown to have important consequences in terms of oxygen delivery and the maintenance of a functional microcirculation manifested by the presence of a near normal FCD. In this study, AVP decreased blood flow but not to the extent where oxygen consumption by the vessel wall significantly reduced tissue oxygenation because this did not fall \(< 8 \text{ mmHg}, \) a value still considerably \( > 2.3–2.8 \text{ mmHg} \) which is considered to be the threshold for the start of anaerobic metabolism (21). Furthermore, arterioles in the AVP-treated group were exposed to flowing blood whose \( \text{PO}_2 \) was \( \geq 28 \text{ mmHg} \), thus the arteriolar endothelium was never in hypoxic conditions. Venules and \( \sim 10\% \) of all arterioles vasodilated leading to the decrease in transmural vessel wall \( \Delta \text{PO}_2 \), and presumably vessel wall oxygen consumption, further supporting the hypothesis that vessel wall oxygen consumption is a parameter directly related to microvascular tone. These findings show why treatment with AVP can lead to severe ischemia because it restricts oxygen supply to the tissue by vasoconstriction and by increasing oxygen consumption by the microcirculation.

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