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Microvascular Oxygen Delivery and Interstitial Oxygenation during Sodium Pentobarbital Anesthesia

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Background: **Anesthesia may represent a considerable bias in experimental medicine, particularly in conditions of stress (such as hemorrhage). Sodium pentobarbital (PB), widely used for cardiovascular investigations, may impair oxygen delivery by hemodynamic and respiratory depression. The critical issue, however, is whether the microcirculation can still maintain tissue oxygenation during anesthesia. To answer this question, the authors studied the effect of PB anesthesia on subcutaneous microvascular oxygen delivery and interstitial oxygenation in Syrian golden hamsters.**

Methods: **Sodium pentobarbital anesthesia was induced by intravenous injection (30 mg/kg body weight) and maintained by a 15-min infusion (2 mg · kg^{-1} · min^{-1}), with animals breathing spontaneously (PB-S) or ventilated with air (PB-V). Systemic parameters evaluated were mean arterial pressure (MAP), heart rate, cardiac index (CI), arterial oxygen tension (Pa_{O_2}), arterial carbon dioxide tension (Pa_{CO_2}), base excess, and *p*H. Microvascular and interstitial oxygen tension (P_{O_2}), vessel diameter, red blood cell velocity (v_{RBC}), and blood flow (Q_b) were measured in a dorsal skinfold preparation. Microcirculatory P_{O_2} values were determined by phosphorescence decay.**

Results: **Sodium pentobarbital anesthesia significantly decreased CI, MAP, v_{RBC}, and Q_b. During PB infusion, Pa_{O_2} values were 56 ± 12.8 mmHg (PB-S) and 115.9 ± 14.6 mmHg (PB-V) compared with 69.4 ± 18.2 mmHg and 61.4 ± 12.6 mmHg at baseline. However, microvascular P_{O_2} was reduced by 25–55% in both groups, resulting in an interstitial P_{O_2} decrease from 23.9 ± 5.6 mmHg (control) to 13.1 ± 9.1 mmHg (PB-S) and 15.2 ± 7 mmHg (PB-V). Microcirculatory P_{O_2} values were restored 30 min after PB infusion, even though hemodynamic depression and a light anesthetic plane were maintained.**

Conclusions: **Sodium pentobarbital anesthesia caused impairment of microvascular oxygen delivery and interstitial oxygenation, effects that were not prevented by mechanical ventilation. Although these effects were restricted to deep anesthetic planes, prolonged hemodynamic depression suggests that caution is warranted when using PB as an anesthetic in cardiovascular investigations. (Key words: Acid-base state: base excess; *p*H. Anesthetics, intravenous: sodium pentobarbital. Blood gases: P_{O_2}; P_{CO_2}. Hemodynamics: mean arterial pressure; blood flow, velocity; cardiac index. Measurement techniques: phosphorescence decay, thermodilution. Microcirculation: arterioles; venules; capillaries. Oxygen: oxygen delivery, interstitial, microvascular).**

CONTROVERSY persists regarding the extent to which the use of anesthesia represents a considerable bias in experimental medicine. Although the use of volatile or nonvolatile anesthetics is often inevitable because of the invasiveness of experimental procedures needed to provide access to inner body tissues or organs, previous investigations have shown that anesthesia may substantially alter experimental conditions, resulting in relatively greater pathophysiologic effects, when a severe stress such as hemorrhage is induced.[1–3] Similarly, in the clinical setting, use of anesthesia may compromise outcomes in conditions of shock, severe trauma, or critical illness.[4]

Barbiturates are commonly used as anesthetic agents both in clinical and experimental conditions. However, in experimental medicine, in contrast to clinical practice, barbiturates are often used as monoanesthetics to provide surgical anesthetic planes and to maintain anesthesia for extended periods of a protocol.[5,6]

Sodium pentobarbital (PB), an oxybarbiturate, is used in as many as 50% of cardiovascular investigations involving both small and large animal species.[5] Previous research has shown that PB causes myocardial[7–10] and respiratory depression,[11–13] direct alterations of the vas-

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culature at the macro- and microcirculatory levels,[14-16] and interference with neural and humoral cardiovascular control mechanisms.[2,9,10,17,18] The occurrence and the extent of these side effects, however, vary.[19]

Sodium pentobarbital anesthesia may also substantially compromise oxygen delivery at the systemic[20] and organ levels[21] due to changes in the cardiovascular and respiratory systems. This appears to be particularly true when severe perturbations, such as hemorrhage or extensive ischemia, are induced, even if such supportive therapeutic measures as mechanical ventilation or oxygen insufflation are used to preserve systemic oxygen uptake.[20,21] Although the impact of PB anesthesia on systemic oxygen delivery, a function of oxygen content and blood flow (cardiac output [CO]), can be assessed accurately, this may not be indicative of oxygen delivery at the microcirculatory level, where local and central regulatory mechanisms may differently alter blood flow and the actual amounts of oxygen delivered to individual tissues.

Our intention was to assess the effects of PB anesthesia on subcutaneous microvascular oxygen delivery and interstitial oxygenation in the hamster skinfold preparation,[22] a long-term animal model that avoids the effects of acute surgical stress and major systemic perturbations. The phosphorescence decay method provides the unique opportunity to make a detailed analysis of oxygen tension in arterioles and venules of different sizes and branching orders[23] in the microvascular network of this preparation, coupled with the ability to assess related interstitial oxygenation.[24-26]

We simultaneously evaluated macro- (mean arterial pressure [MAP], heart rate, cardiac index [CI]) and microhemodynamic (vessel diameter, red blood cell [RBC] velocity, and blood flow) conditions and respiratory and metabolic parameters to show the extent to which microvascular oxygen delivery and resulting interstitial oxygenation would be affected by alterations at the systemic or microcirculatory level introduced with this type of anesthesia. We made measurements in animals breathing spontaneously or mechanically ventilated with air during anesthesia to address the effect of respiratory depression on the regulation of microcirculatory partial pressure of oxygen (P_{O_2}) levels.

We induced PB anesthesia by intravenous injection and subsequently maintained it by continuous infusion, a procedure providing controlled and reproducible levels of anesthesia. We analyzed systemic and microcirculatory parameters during and in various intervals after discontinuation of PB infusion to determine the extent to which this type of anesthesia would compromise microvascular oxygen delivery and interstitial oxygenation during different anesthetic planes and after a recovery period.

Materials and Methods

Experimental Model

We analyzed the effects of PB anesthesia on systemic and microcirculatory parameters in male Syrian golden hamsters (50-70 g body weight) fitted with a dorsal skinfold chamber window preparation,[22] which provided microscopic access to the vasculature of subcutaneous skeletal muscle. Chamber implantation and catheterization of the carotid artery and jugular vein were performed under PB anesthesia (50 mg/kg body weight injected intraperitoneally; Nembutal, Abbott Laboratories, North Chicago, IL), and experiments were done after a recovery period of 2-3 days after surgical procedures. Only animals with a healthy appearance and integrity of the skinfold preparation (as evident by the absence of inflammation, bleeding, edema, and low flow states) were used in investigations.

Induction and Maintenance of Anesthesia

For each experiment, anesthesia was induced by an initial intravenous injection of PB (30 mg/kg body weight) from a 10-mg/ml stock solution made from a 50-mg/ml commercial preparation (Nembutal) by dilution with 0.9% sodium chloride (Elkins-Sinn, Cherry Hill, NJ). The PB injection rate was 1 mg/min, which resulted in an induction time of approximately 90-120 s. After 5 min, a continuous infusion of PB at a rate of 2 $mg \cdot kg^{-1} \cdot min^{-1}$ was started and continued for 15 min to maintain anesthesia. As previously described for rats,[6,27] anesthesia depth and duration were assessed by the judgment of consciousness, motor activity, muscle tone, and the responses to nonaversive (palpebral, corneal, and jaw reflex) and noxious stimulation. To evaluate noxious stimulus perception, an alligator clip producing 50 g/mm^2 force was attached for 2 s to the animals' abdomens, tails, and hind paw toes. Any withdrawal or other movements were recorded as positive for nociception.

To investigate the impact of respiratory depression on microvascular and interstitial P_{O_2} levels, animals were divided into two groups, one group spontaneously breathing room air throughout experimentation, and the other group of animals tracheally cannulated and subsequently mechanically ventilated with air (F_IO_2 = 0.21). Tracheal cannulation was performed after intra-

venous PB injection by inserting a small tube (PE 190) into the upper part of the trachea. After tracheal cannulation, animals were immediately connected to a respirator (Rodent Respirator, Harvard Apparatus, Millis, MA) for normal ventilation. Controlled ventilation was performed during PB infusion and for 30 min afterward. Thereafter experimentation was discontinued, because animals began breathing against the respirator and showed symptoms of discomfort.

Evaluation of Systemic Parameters

Mean Arterial Pressure and Heart Rate. Mean arterial pressure was monitored continuously using a carotid artery catheter connected to a transducer linked to an analog recording system (Beckman R611, Beckman Instruments, Schiller Park, IL). Heart rate was determined both from chart recordings and with a digital monitor specialized for high heart rates (J&MO Technologies, San Diego, CA).

Cardiac Output and Cardiac Index. Cardiac output measurements were performed in a separate group of ten anesthetized animals spontaneously breathing room air throughout the experiment. These animals were larger in size and body weight (150–200 g) than the animals used to analyze microcirculatory parameters, a technical requirement for the thermodilution technique[28] used. Cardiac output measurements involved placement of a 1-Fr thermoprobe (Columbus Instruments, Columbus, OH) into the aortic arch *via* the right carotid artery, which was not feasible in smaller animals. Cardiac output was calculated by a thermodilution CO computer (Cardiotherm 500, Columbus Instruments) that continuously recorded aortic blood temperature and the exact temperature of the 0.175-ml volume of 0.9% sodium chloride injectate (15–20°C), providing an instantaneous digital CO reading of the following saline injection. To provide comparable experimental conditions, animals used for CO measurements also had a dorsal skinfold window installed before experimentation, whereas a PE-50 catheter was inserted into the left jugular vein. For CO experiments, initial intravenous PB injection was performed at a rate of 3 mg/min to maintain the induction period of 90–120 s used in the animals weighing 50–70 g.

Cardiac output was not measured in ventilated animals, because basic macro- and microhemodynamic parameters (MAP, heart rate, microvessel diameter, microvascular blood flow, and RBC velocity) were not substantially different from animals spontaneously breathing air. To exclude the possibility of significant changes in hemodynamics and body temperature related to the repeated cold saline injections over time and the volume of PB injection and infusion, CO was also determined (during a period of 24 h) in five animals that had 0.9% sodium chloride (NaCl) infused. For data presentation, CO (ml/min) was converted into CI ($ml \cdot min^{-1} \cdot kg^{-1}$) accounting for the animals' body weights.

Blood Gases, Base Excess, *p*H, Hemoglobin, and Hematocrit Concentration. Blood samples for determination of arterial P_{O_2}, arterial carbon dioxide tension (P_{CO_2}), base excess and *p*H (Ciba Corning 238 *p*H/Blood Gas Analyzer, Ciba Corning Diagnostics, Pleasanton, CA), hemoglobin (B-Hemoglobin Photometer Hemocue AB, Ängelholm, Sweden), and hematocrit concentrations (Readacrit Centrifuge, Clay Adams Division of Becton, Dickinson, Parsippany, NJ) were collected in 40-μl heparin-washed microtubes (Microhematocrit Capillary Tubes, Scientific Products, Division of Travenol Laboratories, McGraw Park, IL). All blood samples were analyzed immediately and the animals' prevailing central body temperatures were considered when determining blood gas and metabolic parameters.

Body Core and Skin Temperature. Body core temperature was measured rectally and dorsal skin temperature was assessed epidermally in three different locations of the outside skinfold preparation area using a microthermometer (model BAT-12, Physitemp Instruments, Clifton, NJ). In animals in which CO measurements were performed, body core temperature was measured continuously with the aortic thermoprobe. Ambient room temperature was maintained at 25°C during the experiments.

Evaluation of Microhemodynamic Parameters

Intravital Microscopy. Microvessels in the subcutaneous skeletal skin muscle were observed with an inverted microscope (IMT-2, Olympus, New Hyde Park, NY) using a 40× water immersion objective (Olympus WPlan, NA = 0.7) and transillumination technique. Microscopic images were recorded using a camera (Cohu 4815-2000) and transferred to a TV-VCR (Sony Trinitron PVM-1271Q monitor [Tokyo, Japan] and Panasonic AG-7355 video recorder [Tokyo, Japan]).

Microvessel Classification. Before experimentation, microvessels were classified according to branching order in light modification of a nomenclature previously used for the microvasculature in rat skeletal muscle.[23] Arterioles were grouped into large feeding arterioles (A1), small arcading arterioles (A2), transverse arterioles (A3), and terminal arterioles (A4). Venules were divided into two groups, small collecting (Vc) and large venules (V1). Capil-

laries and interstitial (tissue) segments (Int) were supplied and drained by the selected arteriolar and venular tree, thus forming a functional unit with these microvessels.

Microvessel Diameter and Red Blood Cell Velocity. Microvessel diameters and RBC velocities were analyzed on-line in arterioles and venules. Vessel diameter[29] was measured with an image-shearing system (Digital Video Image Shearing Monitor, model 908, I.P.M., Inc., San Diego, CA), and RBC velocity[30] was analyzed by photodiodes (Fiber Optic Photo Diode Pickup System, I.P.M., Inc.) and the cross-correlation technique (Velocity Tracker Mod-102 B, I.P.M., Inc.). In capillaries, RBC velocity was measured off-line from video recordings using dual-window and cross-correlation techniques.

Microvascular Blood Flow. Blood flow (Q_b) in arterioles and venules was calculated from microvessel diameters (d) and RBC velocity (υ_{RBC}) readings according to the formula

$$Q_b = \upsilon_{RBC} \cdot \pi \cdot d^2/4.$$

Functional Capillary Density. Functional capillary density was analyzed on-line from four to six video-recorded microscopic fields exhibiting three to six different capillaries each. It was defined as the number (percentage) of perfused capillaries in which functionality was assumed only if there was RBC flow over the entire capillary length (400–800 μm). We selected 15–20 different capillaries supplied from different terminal (A4) arterioles branching from the selected transverse (A3) arteriole. Because each A3 arteriole feeds 60–80 capillaries, measurements covered approximately 20–25% of anatomic capillary density related to this blood vessel. This corresponds to a tissue area of about 0.24–0.96 mm^2, which is 0.3–1.2% of the entire skinfold preparation area.

Analysis of Microvascular and Interstitial P_{O_2}

Subcutaneous microvascular and interstitial P_{O_2} were determined with the previously described phosphorescence decay technique.[24–26] Based on the oxygen-dependent quenching of phosphorescence emitted by albumin-bound metalloporphyrin complexes after pulsed light excitation, the method allows noninvasive assessment of intravascular and interstitial P_{O_2}, because intravascularly injected porphyrin complexes extravasate into the interstitium over time. The relation between phosphorescence lifetime τ and oxygen tension is given by the Stern-Volmer equation

$$\tau_0/\tau = 1 + kq \cdot \tau_0 \cdot pO_2$$

where τ_0 and τ(μs) are the phosphorescence lifetimes in the absence of molecular oxygen and at a given P_{O_2}, respectively, and kq (Torr $\cdot$ s^{-1}) is the quenching constant, both factors being pH and temperature dependent.

Palladium-meso-tetra (4-carboxyphenyl) porphyrin (Porphyrin Products, Logan, UT) after previous binding to serum albumin and dilution in sodium chloride 0.9% (Elkins-Sinn) to a concentration of 15 mg/ml was used as a phosphorescent probe (τ_0 = 600 μs, kq = 325 Torr/s at pH 7.4 and 37°C) and intravenously injected (30 mg/kg body weight).

Phosphorescence was excited by light pulses (30 Hz) generated by a 45-W xenon strobe arc (EG&G Electro Optics, Salem, MA), whereas the P_{O_2} measuring site was microscopically vignetted by an adjustable slit. For microvascular P_{O_2} measurements, the slit was longitudinally fitted within the vessel. Oxygen tension in the interstitium was measured in eight to ten (parallel) intercapillary segments, where the selected slit size was approximately 10 × 40–60 μm, strictly avoiding spatial interference with or close proximity (<10 μm) to any blood vessel. Filters of 420 and 630 nm were used for porphyrin excitation and phosphorescence emission, respectively. Phosphorescence signals were captured by a photomultiplier (EMI, 9855B, Knott Elektronik, Munich, Germany). Using a digital oscilloscope (Hitachi Oscilloscope V-1065, 100 MHz; Hitachi Denshi, Tokyo, Japan), 128 decay curves were averaged, visualized, and saved. Decay time constants were determined by computer fitting the averaged decay curves to a single exponential,[26] using the Stern-Volmer equation and predetermined parameters of τ_0 and k_q corrected for the prevailing systemic blood pH and the temperature in the window chamber. Because we did not measure interstitial pH and it cannot be precluded that these values were slightly lower than systemic blood pH levels, interstitial P_{O_2} values may be slightly overestimated, because τ_0 decreases and k_q increases on the acidic side of pH 7.2 (17% and 8%, respectively, between pH 6.8 and 6.2).[25]

Although initial studies with albumin-bound palladium-porphyrin did not show consistent extravasation of the dye into the interstitium over time,[26] subsequent refinements of the technique permitted fewer light pulses and an increased observation period without evidence of vasoconstriction and revealed the presence of the porphyrin compound in the interstitium. The reflection coefficient for albumin in subcutaneous tissue ranges from 0.8–0.9, causing natural and continuous extravasation, which in this preparation yields a measurable (steady-state) concentration of the albumin-bound

porphyrin complex about 20–30 min after dye injection. The dye is evenly distributed in the interstitium, because there is no evidence for significant variability in signal strength when no underlying blood vessels are present. In addition, size of the sampled (interstitial) volume, which is determined by the adjustable slit, does not significantly alter P_{O_2} readings as long as there is no spatial interference with the adjacent microvasculature. In these experiments, all microvascular and interstitial P_{O_2} measurements were performed within 5–10 min after the initial equilibration period of 20–30 min after dye injection.

Experimental Design and Statistics

Systemic and microcirculatory effects of PB anesthesia were investigated in 42 animals, with 28 animals spontaneously breathing (PB-S) and 14 animals tracheally cannulated and mechanically ventilated with air (PB-V) during experimentation. In nonventilated animals, repeated measurements of systemic and microhemodynamic parameters were performed before, during PB infusion, and at 30-min, 90-min, and 24-h intervals thereafter. The effect of PB anesthesia on CO and CI was investigated in a separate group of 10 (nonventilated) animals in identical intervals. In ventilated animals, experiments were discontinued 30 min after PB infusion.

In both anesthetized groups (PB-S and PB-V), microvascular and interstitial P_{O_2} values were analyzed in groups of seven animals independently for each time point during and after PB infusion, respectively. Systemic and microhemodynamic parameters in these animals did not differ statistically relative to each measuring time point. Repeated P_{O_2} measurements over time in the same animals were avoided to exclude microcirculatory side effects due to excessive light exposure and resulting porphyrin excitation. Control P_{O_2} measurements were assembled in an independent group of seven animals.

To exclude the possibility of significant time-related changes due to the volume of solution required for the PB infusion, blood sampling or deterioration of the preparation, systemic and microhemodynamic parameters were also investigated in a group of 14 animals (NaCl) that received an identical quantity of 0.9% sodium chloride. Repeated microvascular and interstitial P_{O_2} measurements over time were not performed in these animals, because there was no evidence of significant time variability in hemodynamic, respiratory, or metabolic parameters. Repeated measurements of CO and CI were also performed in a group of five animals that received infusions of 0.9% NaCl.

Data are shown as means ± SDs. Because this study was to show time-related changes due to alterations in anesthesia depth and state of consciousness, all statistical comparisons within the different groups (PB-S, PB-V, and NaCl) were made by repeated measures analysis of variance and *post hoc* tests whenever indicated. Statistical comparisons between the PB-S and PB-V groups were also made by analysis of variance. For all statistical tests, 5% ($P < 0.05$) was the minimum level of significance considered.

Results

After sporadic incidences of excitation, shown by vigorous body movements,[6] animals rapidly lost consciousness during initial PB injection. A surgical anesthetic plane adequate for moderately painful procedures was present throughout the following infusion period. This could be concluded (in nonventilated animals) from the response to nonaversive and noxious stimulation indicating the absence of muscle tone, the palpebral and jaw reflex, and tolerance of abdominal and tail pinches.[6,27] It was also shown in the group of ventilated animals that tolerated tracheal cannulation and subsequent mechanical ventilation without evidence of symptoms of pain or distress. A light anesthetic plane adequate for minimally painful interventions was still maintained 30 min after discontinuation of PB infusion. Shortly after this point, ventilated animals began to fight the respirator and showed symptoms of discomfort. Nonventilated animals, which were followed for 24 h, all regained consciousness and normal motor activity 90 min after PB infusion. Recovery from anesthesia was associated with sporadic occurrences of animal excitation.[6] Even though the animals' airways were not secured by tracheal cannulation and no supplemental oxygen was provided, the experimental procedure was tolerated very well, as evident by normal sleeping and feeding habits and normal motor activity 24 h after the experiments.

Sodium pentobarbital anesthesia caused substantial macrohemodynamic depression as shown by decreases in CO, CI, and MAP (fig. 1). In nonventilated animals, CO and CI decreased by 22% from 60.8 ± 9.4 ml/min and 330.9 ± 24.1 $ml \cdot min^{-1} \cdot kg^{-1}$ at baseline (Bsln) to 47.4 ± 8.3 ml/min and 257.4 ± 20.6 $ml \cdot min^{-1} \cdot kg^{-1}$ during PB infusion (Inf). Cardiac output and CI readings (Bsln) were in a range previously reported for this ani-

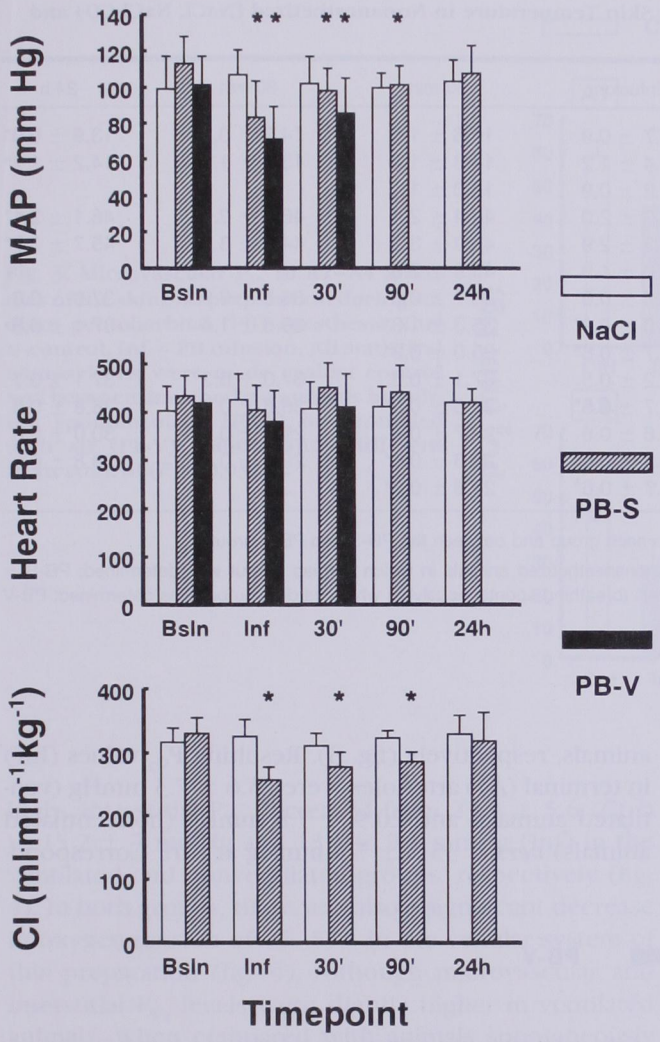

Fig. 1. Mean arterial pressure (MAP), heart rate, and cardiac index (CI) during sodium pentobarbital (PB) anesthesia. Bsln = baseline. Inf = PB infusion. All statistical comparisons were made against baseline in each group (anesthetized animals breathing spontaneously [PB-S] or ventilated with air [PB-V] and nonanesthetized animals [NaCl] receiving 0.9% sodium chloride) and between the PB-S and PB-V groups. *Significantly different from baseline ($P < 0.05$).

mal species.[31] Mean arterial pressure decreased from 113.2 ± 14.7 mmHg (Bsln) to 83.1 ± 20.3 mmHg (Inf) in animals spontaneously breathing air, whereas MAP decreased from 101.7 ± 15.5 mmHg (Bsln) to 71.1 ± 18.1 mmHg (Inf) in ventilated animals. Cardiac output and CI were not determined in ventilated animals, because basic macro- and microhemodynamic parameters were not significantly different from those in animals spontaneously breathing air. Although CO, CI, and MAP increased during the recovery period, values did not return to normal levels 90 min after PB infusion. Induction of PB anesthesia was associated with a light, but not significant, increase in MAP and heart rate (not shown in figure 1). Heart rates declined relatively during PB infusion, but changes were not significant. Macrohemodynamic parameters (CO, CI, MAP, and heart rate) did not change significantly over time in animals that received 0.9% NaCl (fig. 1).

Hemoglobin and hematocrit concentrations did not change significantly during PB infusion and after 30 min but were slightly, although significantly ($P < 0.05$), decreased in nonventilated animals 90 min (13.9 ± 1.4 g/dl and 44 ± 3.2%) and 24 h (14.2 ± 0.8 g/dl and 45.7 ± 1.7%) after infusion (table 1). A comparable reduction in both parameters was observed in nonanesthetized animals.

Sodium pentobarbital anesthesia also caused a significant ($P < 0.05$) decrease in rectal and skin temperatures in all animals, which were approximately 2°C and 1°C, respectively (table 1). In anesthetized animals in which CO and CI were determined, aortic temperature decreased by ≈1.5°C during PB infusion and after 30 min. Normal temperature levels were sustained during the entire 24-h observation period in animals receiving 0.9% NaCl.

Sodium pentobarbital anesthesia caused significant ($P < 0.05$) respiratory depression in nonventilated animals, as shown by the decrease in systemic arterial P_{O_2} values ± 18.2 mmHg (Bsln) to 56 ± 12.8 mmHg (Inf) and the increase of P_{CO_2} values from 43.8 ± 8.4 mmHg (Bsln) to 57.7 ± 9.1 mmHg (Inf) (fig. 2). These changes were associated with a decline in arterial blood *p*H from 7.37 ± 0.05 (Bsln) to 7.22 ± 0.05 (Inf). This reduction of blood *p*H exhibited predominantly respiratory acidosis, because base excess only slightly decreased from 1.8 ± 3.8 mM (Bsln) to −1.8 ± 3.4 mM (Inf). This decline persisted during the following 90 min. Relative increase of P_{O_2} and decrease of P_{CO_2} levels at that time were indicative of a tendency of hyperventilation; changes, however, not reaching significance.

In ventilated animals, systemic arterial P_{O_2} values increased from 61.4 ± 12.6 mmHg (Bsln) to 115.9 ± 14.6 mmHg (Inf) and 109.0 ± 16.1 mmHg during the following 30-min period (fig. 2). Although P_{CO_2} values and *p*H (46.1 ± 7.1 mmHg and 7.36 ± 0.05 at Bsln) were maintained at normal levels in these animals, base excess decreased from 3.3 ± 2.5 mM (Bsln) to 0.2 ± 2 mM (Inf) and −0.4 ± 2.1 mM at 30 min. In saline-infused animals, blood gas and metabolic parameters did not change significantly over time.

At the microcirculatory level, PB anesthesia caused a significant reduction of oxygen tension in arterioles and

Table 1. Hemoglobin Concentration, Hematocrit, Body Core, and Skin Temperature in Nonanesthetized (NaCl, NaCl-CO) and Sodium Pentobarbital-anesthetized Animals (PB-S, PB-SCO, PB-V)

Parameter	Group	Baseline	Infusion	30 min	90 min	24 h
Hemoglobin (g/dl)	NaCl	15.2 ± 1.1	14.7 ± 0.9	14.6 ± 1.2	14.3 ± 0.7	13.9 ± 0.9*
	PB-S	15.0 ± 0.7	14.4 ± 1.2	14.8 ± 1.0	13.9 ± 1.4*	14.2 ± 1.2*
	PB-V	14.7 ± 0.7	14.2 ± 0.9	14.0 ± 1.1		
Hematocrit (%)	NaCl	48.3 ± 2.3	47.3 ± 2.0	46.4 ± 2.4	46.3 ± 2.5*	46.1 ± 2.1*
	PB-S	47.3 ± 2.4	46.3 ± 2.9	46.9 ± 3.6	44.0 ± 3.2*	45.7 ± 3.7*
	PB-V	47.3 ± 2.1	46.0 ± 1.9	45.9 ± 2.5		
Rectal temperature (°C)	NaCl	37.0 ± 0.6	36.8 ± 0.6	36.7 ± 0.5	36.8 ± 0.9	37.0 ± 0.6
	PB-S	37.3 ± 0.6	35.0 ± 0.9*	35.3 ± 1.8*	36.6 ± 1.4	37.1 ± 0.8
	PB-V	36.9 ± 0.7	34.7 ± 0.8*	35.0 ± 0.9*		
Aortic temperature (°C)	NaCl-CO	37.4 ± 0.5	37.2 ± 0.5	37.1 ± 0.3	37.0 ± 0.3	37.1 ± 0.7
	PB-SCO	37.1 ± 0.5	35.7 ± 0.6*	35.6 ± 0.9*	36.6 ± 0.7	36.8 ± 0.6
Skin temperature (°C)	NaCl	29.9 ± 0.5	29.6 ± 0.6	29.7 ± 0.4	29.8 ± 0.4	30.0 ± 0.6
	PB-S	30.2 ± 0.4	29.2 ± 0.5*	29.3 ± 0.6*	29.5 ± 0.7	29.8 ± 0.5
	PB-V	29.7 ± 0.7	28.7 ± 0.6*	28.8 ± 0.8*		

Values are mean ± SD. All statistical comparisons were made against baseline in each group and between the PB-S and PB-V groups.

NaCl = nonanesthetized animals receiving 0.9% sodium chloride; NaCl-CO = nonanesthetized animals in which cardiac output was determined; PB-S = anesthetized animals breathing air spontaneously; PB-SCO = anesthetized animals (breathing spontaneously) in which cardiac output was determined; PB-V = anesthetized animals ventilated with air.

* Significantly different from baseline ($P < 0.05$).

venules and in the interstitium of this preparation. In feeding (A1) arterioles, P_{O_2} values declined from 58.6 ± 4.9 mmHg at control (Ctrl) to 46.8 ± 4.9 mmHg and 43.2 ± 10.4 mmHg (Inf) in ventilated and nonventilated animals, respectively (fig. 3). Resulting P_{O_2} values (Inf) in terminal (A4) arterioles were 23.6 ± 7.3 mmHg (ventilated animals) and 20.9 ± 7.8 mmHg (nonventilated animals) *versus* 35.2 ± 7.9 mmHg at Ctrl. Correspond-

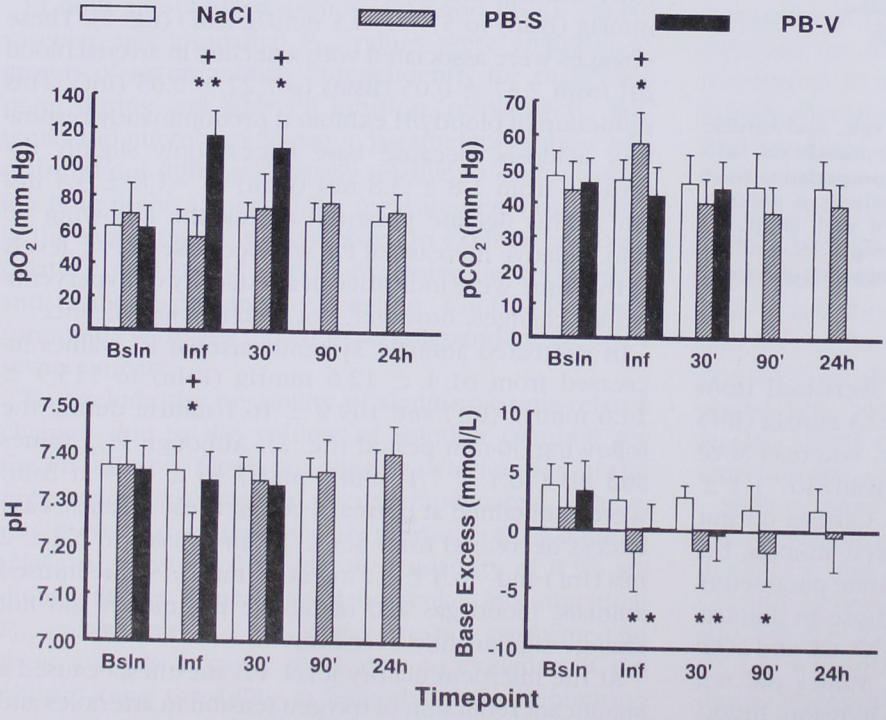

Fig. 2. Arterial blood gases, base excess, and *p*H during sodium pentobarbital (PB) anesthesia. Bsln = baseline. Inf = PB infusion. All statistical comparisons were made against baseline in each group (anesthetized animals breathing spontaneously [PB-S] or ventilated with air [PB-V] and nonanesthetized animals [NaCl] receiving 0.9% sodium chloride) and between the PB-S and PB-V groups. *Significantly different from baseline. **Significantly different between the PB-S and PB-V groups ($P < 0.05$).

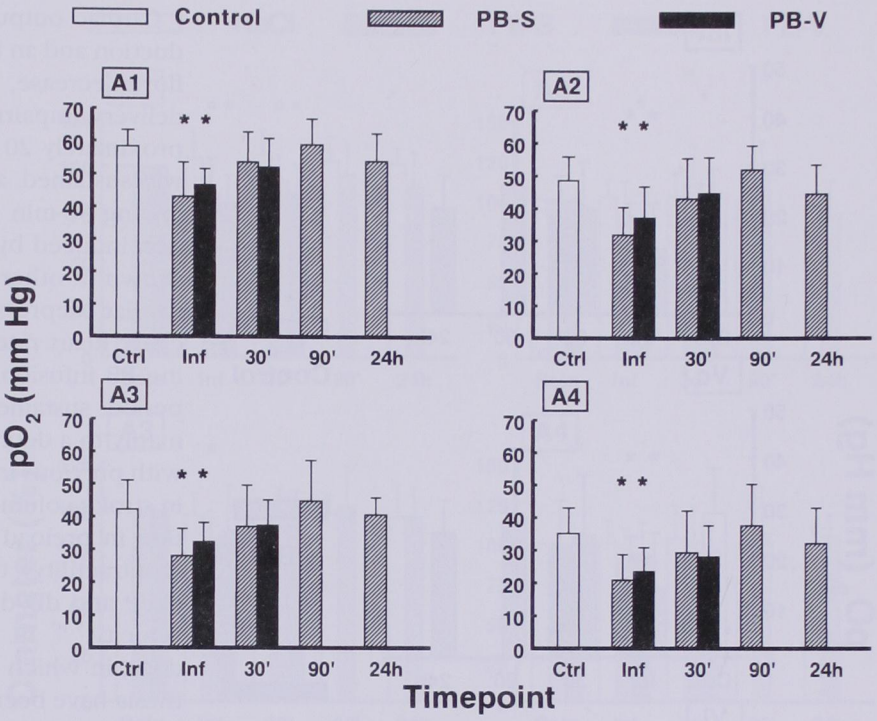

Fig. 3. Microvascular P_{O_2} in A1–A4 arterioles of the skinfold preparation during sodium pentobarbital (PB) anesthesia. Ctrl = control. Inf = PB infusion. All statistical comparisons were made against control and between anesthetized animals breathing spontaneously (PB-S) or ventilated with air (PB-V). *Significantly different from control ($P < 0.05$).

ingly, interstitial P_{O_2} decreased from 23.9 ± 5.6 (Ctrl) to 15.2 ± 7 mmHg and 13.1 ± 9.1 mmHg (Inf) in the ventilated and nonventilated groups, respectively (fig. 4). In both groups, there was also a significant decrease in oxygen tension of 45–55% in the venular system of this preparation (fig. 4). Although microvascular and interstitial P_{O_2} levels were slightly higher in ventilated animals, when compared with animals spontaneously breathing air, these differences were not significant. In both groups, microvascular and interstitial P_{O_2} values were restored to low-normal levels 30 min after PB infusion, an effect that was sustained in nonventilated animals during the following 90-min and 24-h periods.

Sodium pentobarbital anesthesia also caused significant alterations in arteriolar and venular (micro-) hemodynamics. During and 30 min after PB infusion, vessel diameters of feeding (A1) arterioles significantly ($P < 0.05$) decreased in both groups (fig. 5). A significant decline of vessel diameters was also observed in arcade (A2) arterioles in the ventilated and in transverse (A3) arterioles in the nonventilated animals during PB infusion. Microvessel diameters did not change significantly in A4 arterioles and venules and in animals receiving 0.9% NaCl. Vasomotion was not seen in the anesthetized or nonanesthetized groups at any time.

In all anesthetized animals, there was a significant ($P < 0.05$) reduction of microvascular blood flow in the arteriolar and venular system of this preparation, which was approximately 25–55% (fig. 6) and paralleled by a comparable decrease in microvascular RBC velocities (not shown in fig. 6). Blood flow and velocity rates were not significantly different in ventilated and nonventilated animals. Normal values were not restored in all microvessels 30 and 90 min after PB infusion, although blood flow and RBC velocity increased relatively over time. Both parameters were not significantly altered in animals receiving 0.9% NaCl. Baseline microhemodynamics in anesthetized and nonanesthetized animals are summarized in table 2.

During PB infusion, functional capillary density decreased by 15–20%, with no significant difference between ventilated and nonventilated animals (fig. 7). In both groups, capillary RBC velocity declined by 35–45% (Inf) and was not restored to normal levels 30 min thereafter. Normal functional capillary density and capillary RBC velocity were sustained in nonanesthetized animals receiving 0.9% NaCl. Baseline capillary RBC velocities in the anesthetized and nonanesthetized groups are summarized in table 2.

Discussion

The principle finding of this study was that intravenously induced and maintained PB general anesthesia

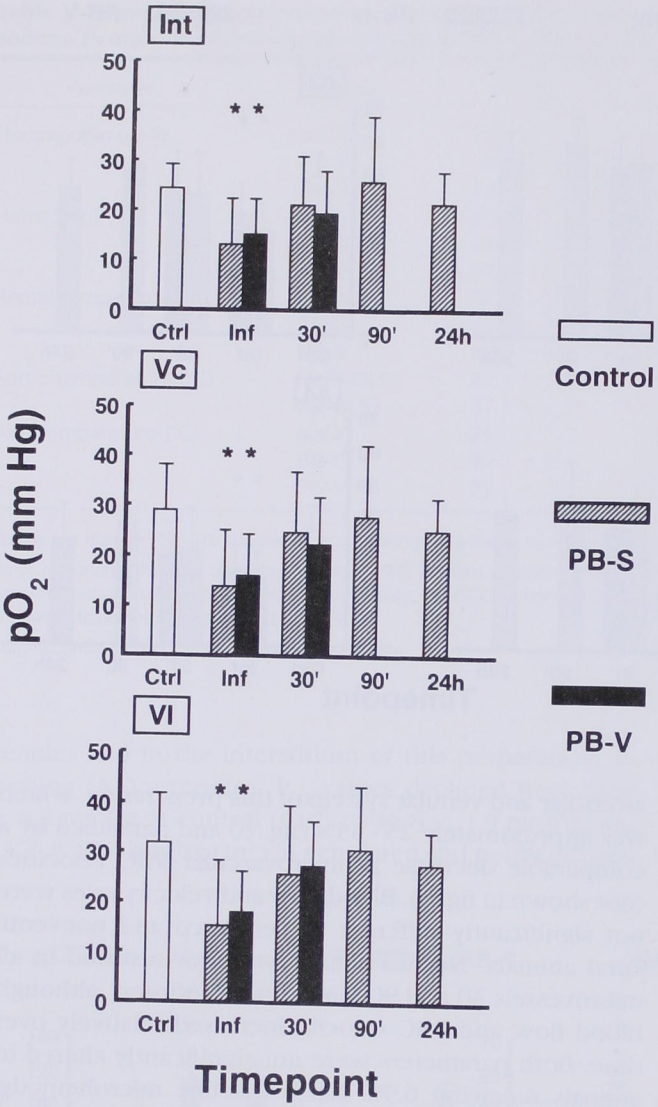

Fig. 4. Interstitial and venular P_{O_2} in the skinfold preparation during sodium pentobarbital (PB) anesthesia. Int = interstitium. Vc = small collecting venule. Vl = large venule. Ctrl = control. Inf = PB infusion. All statistical comparisons were made against control and between anesthetized animals breathing spontaneously (PB-S) or ventilated with air (PB-V). *Significantly different from control ($P < 0.05$).

caused a significant ($P < 0.05$) reduction of microvascular and interstitial P_{O_2} levels in subcutaneous skeletal muscle. This decrease in oxygen tension at the microcirculatory level was not prevented by mechanical ventilation, even though systemic arterial P_{O_2} values were significantly increased. Marked macro- and microhemodynamic depression coincided with a temporary oxygen supply deficit observed in this microcirculatory environment.

Cardiac output depression, a main cause of MAP reduction and an important factor in microvascular blood flow decrease, was a principal mechanism of oxygen delivery impairment. Cardiac output decreased by approximately 20–25% during PB infusion, an effect that was sustained, although attenuated, throughout the following 90-min recovery period. Significant CO reduction induced by this type of anesthesia previously was shown in other animal models, although the extent of cardiac depression may be highly variable.[7–10,19] Because heart rates were not significantly decreased during PB infusion and were normal during the recovery period, sustained CO depression can be attributed primarily to a decrease in stroke volume. This is in accord with previous investigations in which marked decreases in stroke volume were found[7–10] and related to a reduction in preload and impaired left ventricular function (contractility), the latter inferred from decreases in (dP/dt)/P and dD/dt—that is, myocardial fiber shortening velocity.[9,10] In contrast to previous investigations in dogs, in which increases in heart rates during PB anesthesia have been referred to vagolytic, baroreceptor, or sympathetic stimulation,[7–10,19] no such alterations were observed in this animal model. The brief increase in heart rate and MAP during induction of anesthesia may reflect a temporary increase in sympathetic activity associated with a lower level of anesthesia.[17]

The PB-induced decrease in CO would appear to account for the MAP reduction, which was of similar time, course, and magnitude. The reduced CO and resulting MAP decrease may constitute an important mechanism for microvascular blood flow reduction observed in this microcirculatory environment. This may also be concluded from the similar time course of changes in these parameters during anesthesia and after the recovery period. Furthermore, a low intravascular driving pressure caused a significant decrease in arteriolar and venular RBC velocities, a result that can also be attributed to the decrease in CO.

Microvascular blood flow impairment exceeded CO and MAP reduction, and there was also a significant ($P < 0.05$) decrease in arteriolar diameters during PB infusion and after the 30-min recovery period. These effects, however, may be due only in part to vasoconstriction caused by increased sympathetic tone and related to the presence of severe hypothermia.[17,32] This is supported by previous investigations in which PB was shown to suppress plasma catecholamine levels and to decrease reflex responses of sympathetic nerve activities controlling different organs in response to stimulation of arterial baro- and chemoreceptors.[2,17,18]

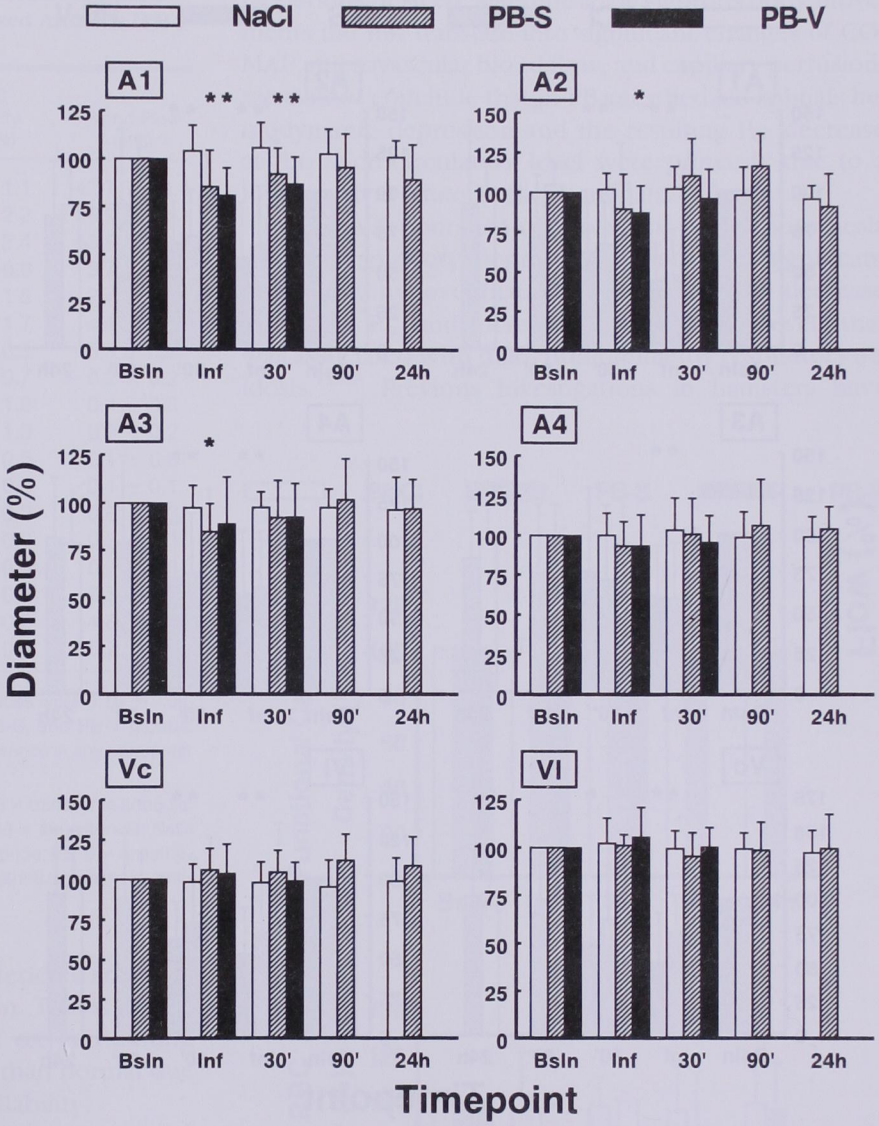

Fig. 5. Diameter changes (%) in arterioles (A1–A4) and venules (Vc and Vl) of the skinfold preparation during sodium pentobarbital (PB) anesthesia. Vc = small collecting venule. Vl = large venule. Bsln = baseline. Inf = PB infusion. All statistical comparisons were made against baseline in each group (anesthetized animals breathing spontaneously [PB-S] or ventilated with air [PB-V] and nonanesthetized animals [NaCl] receiving 0.9% sodium chloride) and between the PB-S and PB-V groups. *Significantly different from baseline ($P < 0.05$). Baseline microvessel diameters in each group are summarized in table 2.

Although these suppressive effects may vary considerably,[17] observed decreases in vessel diameters may also be due to enhanced microvascular tone secondary to the decrease in blood flow and resulting from suppressed release of endothelial flow-dependent relaxing factors.[33,34] Microvascular blood flow impairment may also be, in part, the consequence of a blood flow reduction and redistribution occurring in closer proximity to the systemic circulation, but this could not be visualized in this microcirculatory model.

Findings on microvascular reactivity in this study do not correspond with previous results, in which PB anesthesia caused arterial (arteriolar) and venous (venular) vasodilation,[14-16] attributed to the inhibition of calcium influx through voltage-dependent and receptor-operated channels of plasma membranes.[14,16] Differences in experimental models and superimposition of local and central regulatory mechanisms, however, may explain the discrepancy in results.

Because in all animals microvascular blood flow was reduced by approximately 50-55% in feeding (A1) arterioles, a substantially lesser amount of oxygen than normal was delivered to this microcirculatory environment. This was reflected in a considerable reduction of oxygen tension already in the entrance vessels of this preparation. Decreased oxygen availability in feeding (A1) arterioles also may account for the accentuated decrease in microvascular P_{O_2} values, when progressing

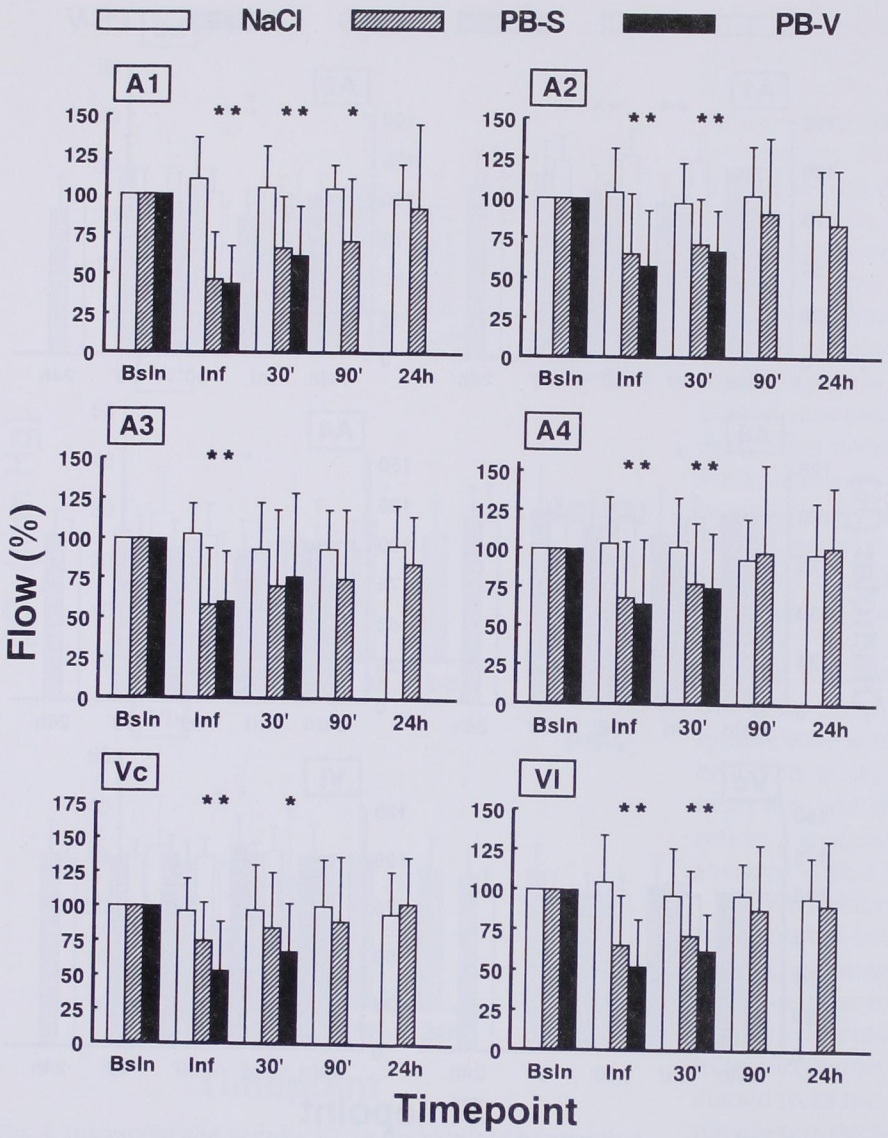

Fig. 6. Blood flow changes (%) in arterioles (A1–A4) and venules (Vc and Vl) of the skinfold preparation during sodium pentobarbital (PB) anesthesia. Vc = small collecting venule. Vl = large venule. Bsln = baseline. Inf = PB infusion. All statistical comparisons were made against baseline in each group (anesthetized animals breathing spontaneously [PB-S] or ventilated with air [PB-V] and nonanesthetized animals [NaCl] receiving 0.9% sodium chloride) and between the PB-S and PB-V groups. *Significantly different from baseline ($P < 0.05$). Baseline blood flow and corresponding red blood cell velocity in each group are summarized in table 2.

from large feeding and arcade (A1, A2) arterioles to subsequent smaller transverse and terminal (A3, A4) arterioles. Although under normal conditions arteriolar oxygen tension decreased by 15% with each branching order (A2, A3, A4), P_{O_2} was reduced by 20–21% in anesthetized animals. This discrepancy in arteriolar P_{O_2} decrease was reflected in the P_{O_2} levels of terminal (A4) arterioles, where under normal conditions 60% of (A1) arteriolar oxygen tension was preserved, whereas it was only 48–50% during anesthesia, indicating that relatively more (≈20%) arteriolar oxygen was unloaded before the capillary circulation. Contributive factors of the augmented exit of arteriolar oxygen may be relatively lower interstitial P_{O_2} levels, determining a larger diffusional gradient, and a significant reduction of microvascular RBC velocity, supporting the concept that the transit time of the oxygen-carrying blood column in contact with the vessel wall determines the amount of oxygen that exits the vascular lumen.[35,36]

We must emphasize that, even under normal conditions, arterioles play a key role in (interstitial) tissue oxygenation by direct oxygen unloading because the vessel wall is a limited barrier to the diffusion of oxygen, having a diffusion constant similar to that of water.[37,38] Even if under the conditions of anesthesia arteriolar oxygen was unloaded relatively faster, this process could not compensate for the oxygen deficit generated at the capillary level, as indicated by significantly de-

Table 2. Baseline Microhemodynamics in Nonanesthetized (NaCl) and Sodium Pentobarbital-anesthetized Animals (PB-S and PB-V)

Vessel Type	Group	Diameter (μm)	RBC Velocity (mm/s)	Blood Flow (nl/s)
A1	NaCl	83.3 ± 31.4	3.7 ± 1.1	42.7 ± 34.4
	PB-S	65.9 ± 20.1	4.5 ± 2.2	36.2 ± 33.1
	PB-V	82.1 ± 13.3	5.1 ± 2.4	53.8 ± 32.1
A2	NaCl	28.4 ± 7.7	3.7 ± 0.8	3.7 ± 1.3
	PB-S	21.4 ± 8.0	3.5 ± 1.5	2.4 ± 2.2
	PB-V	30.6 ± 8.6	3.3 ± 1.7	4.1 ± 3.0
A3	NaCl	11.1 ± 3.1	2.3 ± 0.9	0.3 ± 0.2
	PB-S	9.7 ± 2.6	1.7 ± 0.7	0.2 ± 0.2
	PB-V	11.0 ± 2.8	2.4 ± 1.0	0.4 ± 0.3
A4	NaCl	7.8 ± 2.2	1.6 ± 1.0	0.2 ± 0.2
	PB-S	6.3 ± 2.1	1.0 ± 0.5	0.1 ± 0.0
	PB-V	7.3 ± 2.1	1.5 ± 0.8	0.1 ± 0.1
Vc	NaCl	33.4 ± 6.8	0.5 ± 0.2	0.6 ± 0.3
	PB-S	24.9 ± 7.8	0.4 ± 0.2	0.3 ± 0.3
	PB-V	31.0 ± 9.6	0.5 ± 0.3	0.7 ± 0.6
Vl	NaCl	79.2 ± 24.3	0.6 ± 0.2	7.5 ± 6.5
	PB-S	74.8 ± 28.8	0.5 ± 0.2	4.8 ± 4.7
	PB-V	76.6 ± 19.6	0.6 ± 0.4	5.7 ± 4.0

Values are mean ± SD. Baseline capillary RBC velocities were 0.18 ± 0.08, 0.15 ± 0.08, and 0.21 ± 0.11 mm/s in the NaCl, PB-S, and PB-V groups, respectively. There was not statistically significant difference in any parameter between the different groups ($P < 0.05$).

A1 = feeding arteriole; A2 = small arcading arteriole; A3 = transverse arteriole; A4 = terminal arteriole; Vc = small collecting venule; Vl = large venule; NaCl = nonanesthetized animals receiving 0.9% sodium chloride; PB-S = anesthetized animals breathing air spontaneously; PB-V = anesthetized animals ventilated with air.

creased P_{O_2} values in terminal (A4) arterioles and thus preserve normal interstitial oxygenation. This is due to the fact that the absolute amounts of oxygen exiting the arteriolar vessel lumen are smaller than normal due to decreased intravascular oxygen availability.

Considering that the capillary network represents the largest area of oxygen transfer to the tissue (interstitium), marked decreases (40–45%) in interstitial P_{O_2} values may also be related to a slight decrease in functional capillary density during PB infusion and to substantially reduced capillary RBC velocities, an effect that was sustained throughout the 30-min follow-up period. Capillary perfusion impairment was a consequence of arteriolar blood flow and RBC velocity reduction.

Substantial blood flow impairment during PB anesthesia was also found previously in other vascular beds, including the heart,[3] brain,[3] splanchnic organs,[3,4,9] and skeletal muscle circulation,[3] using different animal models and measuring techniques such as microspheres[3] or the Doppler flow method.[4,9] Considering that in unanesthetized animals different levels of motor activity shown by periods of sleep, sleepiness, or vigorous body movements did not translate into significant changes of CO, MAP, microvascular blood flow, and capillary perfusion, we cannot conclude that in PB-anesthetized animals hemodynamic depression and the resulting P_{O_2} decrease at the microcirculatory level were primarily due to a lower activity state in these animals.

In animals spontaneously breathing air, PB anesthesia caused respiratory depression resulting in significant pulmonary hypoventilation as shown by the decrease in arterial P_{O_2} and increase in arterial P_{CO_2} levels that was associated with mild, predominantly respiratory acidosis.[11–13] Previous investigations in hamsters have

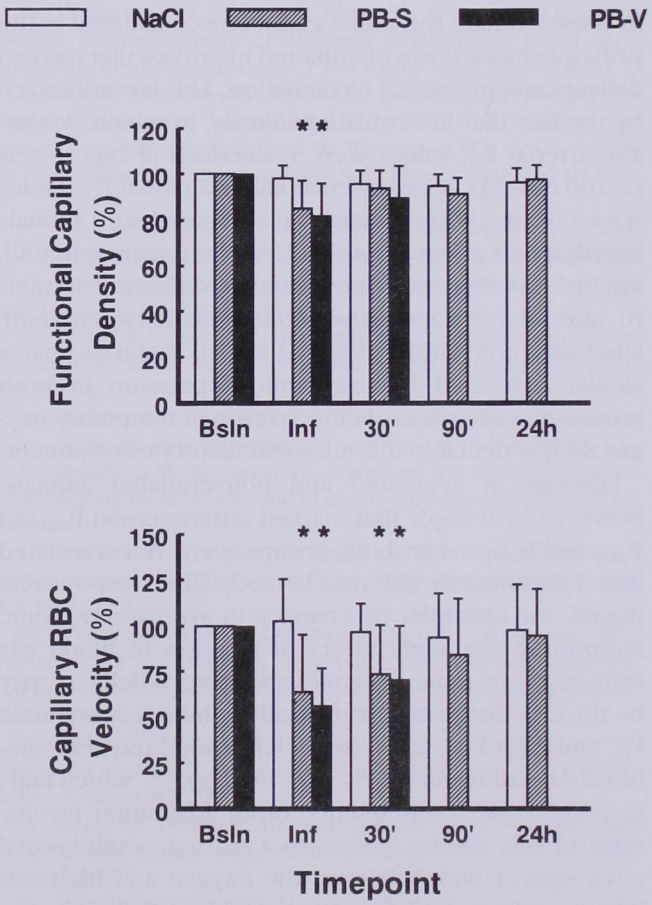

Fig. 7. Changes (%) of functional capillary density (FCD) and capillary red blood cell (RBC) velocity in the skinfold preparation during sodium pentobarbital (PB) anesthesia. Bsln = baseline. Inf = PB infusion. All statistical comparisons were made against baseline in each group (anesthetized animals breathing spontaneously [PB-S] or ventilated with air [PB-V] and nonanesthetized animals [NaCl] receiving 0.9% sodium chloride) and between the PB-S and PB-V groups. *Significantly different from baseline ($P < 0.05$). Baseline capillary RBC velocities in each group are summarized in table 2.

shown that PB anesthesia may substantially alter normal lung and airways mechanics, resulting in significantly decreased lung volume and compliance and in multiple-resistance increase.[13] These factors could substantially compromise pulmonary gas exchange and imply the occurrence of severe arterial hypoxemia, but we did not find this in our study because there was only a moderate decrease in arterial oxygen tension (56 ± 12.8 mmHg compared with 69.4 ± 18.2 mmHg at Bsln). This is a notable finding, because the animals' airways were not secured by tracheal cannulation and no supplemental oxygen was provided.

Although respiratory depression by decreasing arterial blood oxygen content may have contributed to the decreased oxygen availability at the microcirculatory level in these animals, this effect cannot be considered as the principle mechanism of impaired microvascular oxygen delivery and interstitial oxygenation. This is emphasized by the fact that in ventilated animals, in which systematic arterial P_{O_2} values were maintained at high levels (>100 mmHg), microvascular and interstitial P_{O_2} values were not significantly increased compared with animals breathing air spontaneously. This is a remarkable finding because there was no significant difference in macro- and microhemodynamic conditions between ventilated and nonventilated animals. This suggests that a similar extent of hemodynamic depression in both groups was the principle mechanism of temporary oxygen supply deficit in this microcirculatory environment.

Findings in ventilated and nonventilated animals, however, also imply that marked differences in P_{O_2} and P_{CO_2} levels between both groups were not translated into a significantly different hemodynamic response induced, for example, by changes in sympathetic tone. Supporting the slight effect of changes in blood gas state or *p*H on hemodynamics and oxygen delivery may be the fact that hamsters normally exhibit low systemic P_{O_2} and high P_{CO_2} levels and a left-shifted oxyhemoglobin dissociation curve (P_{50} = 28 mmHg),[36,39] which may, in part, be the consequence of an adaptation mechanism of this animal species to a natural, semifossorial environment with inherent low oxygen and high carbon dioxide content.[40]

Although microvascular and interstitial P_{O_2} levels were decreased substantially in this preparation during PB infusion, it cannot be assumed that oxygen delivery impairment was sufficiently severe to cause substantial cellular hypoxia. This is supported by the observation that intact mitochondrial function requires only a P_{O_2} of 0.5 mmHg or less.[41] Furthermore, interstitial P_{O_2} levels were greater than the "critical" extracellular P_{O_2}, which has been reported to be less than 10 mmHg.[42,43] In addition, there was only a slight decrease in base excess levels in all animals, indicating some metabolic blood acidosis. This parameter has been shown previously to be an accurate indicator of whole-body oxygen debt[44] and to correlate well (although not determined in this study due to the animals' small blood volume) with blood lactate levels.[45]

In all animals, microvascular and interstitial P_{O_2}s were restored to normal levels 30 min after PB infusion was discontinued, even though a light anesthetic plane, adequate for minimal invasive procedures, was maintained. A relative increase in systemic arterial P_{O_2} values to normal levels, however, may not be the principle mechanism of microcirculatory P_{O_2} recovery in nonventilated animals, because in ventilated animals, under comparable hemodynamic conditions, high systemic P_{O_2}s were sustained. Therefore, recovery of microvascular and interstitial P_{O_2} levels may be primarily related to a relative improvement in macro- and microhemodynamics, although normal conditions were not yet restored. Persisting hemodynamic depression may explain why microvascular and interstitial P_{O_2} levels were still in a "low normal" range at that time. In nonventilated animals, which were followed for 24 h, slight hemodynamic depression was maintained 90 min after PB infusion was discontinued, even though all animals regained consciousness and exhibited normal motor activity. This finding further emphasizes that changes in hemodynamics and oxygen delivery during PB anesthesia cannot be related primarily to a lower level activity state but must be attributed to direct cardiovascular effects of this anesthetic agent.

The effect of PB on hemodynamics and oxygen delivery must be considered carefully in studies in which this drug is used as a sole anesthetic, particularly in conditions of stress[2,3] but also in investigations in which other intravenous or volatile anesthetics or cardiovascular drugs are used that may interfere or reinforce effects.[10,15]

In conclusion, PB anesthesia caused significant impairment of microvascular oxygen delivery and interstitial oxygenation in the hamster skinfold preparation. These effects were related to marked macro- and microhemodynamic depression as shown by significant decreases in CO, MAP, and microvascular blood flow. Controlled ventilation, increasing arterial blood oxygen content, did not significantly improve interstitial oxygenation. This suggests that respiratory depression was not a principle mechanism of impaired oxygen delivery. Normal

microvascular and interstitial P_{O_2} levels were restored 30 min after PB infusion was discontinued, even though a light anesthetic plane was maintained. Prolonged macro- and microhemodynamic depression, however, suggest that caution may be warranted when using PB as an anesthetic in cardiovascular investigations (that is, those involving hemorrhage).

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