Am J Physiol Heart Circ Physiol 295: H1090–H1099, 2008.
First published June 20, 2008; doi:10.1152/ajpheart.00303.2008.

Polymerized bovine hemoglobin decreases oxygen delivery during normoxia and acute hypoxia in the rat

David C. Irwin,[1] Ben Foreman,[1] Ken Morris,[1] Molly White,[1,2,3] Tim Sullivan,[1] Robert Jacobs,[3] Eric Monnet,[4] Tim Hackett,[4] Martha C. TissotvanPatot,[2] Karyn L. Hamilton,[3] and Robert W. Gotshall[3]

[1]*Cardiovascular Pulmonary Research Group, School of Medicine;* [2]*Department of Anesthesiology, University of Colorado Health Science Center, Aurora, Colorado;* [3]*Department of Health and Exercise Science; and* [4]*Department of Biomedical Sciences School of Veterinary Medicine, James L. Voss Veterinary Teaching Hospital, Colorado State University, Fort Collins, Colorado*

Submitted 20 March 2008; accepted in final form 17 June 2008

Irwin DC, Foreman B, Morris K, White M, Sullivan T, Jacobs R, Monnet E, Hackett T, TissotvanPatot MC, Hamilton KL, Gotshall RW. Polymerized bovine hemoglobin decreases oxygen delivery during normoxia and acute hypoxia in the rat. *Am J Physiol Heart Circ Physiol* 295: H1090–H1099, 2008. First published June 20, 2008; doi:10.1152/ajpheart.00303.2008.—Hemoglobin-based oxygen carriers (HBOC) have been primarily studied for blood loss treatment. More recently infusions of HBOC in euvolemic subjects have been proposed for a wide variety of potential therapies in which increased tissue oxygenation would be beneficial. However, compared with the exchange transfusion models to study blood loss, less is known about HBOC oxygen delivery and vasoacitvity when it is infused in euvolemic subjects. We hypothesized that HBOC [polymerized bovine hemoglobin (PBvHb)] infusion creating hypervolemia would increase oxygen delivery to tissues during acute global hypoxia. Vascular oxygen content and hemodynamics were determined after euvolemic rats were infused with 3 ml of either lactated Ringer or PBvHb solution (13 g/dl, 1.3 g/kg) during acute hypoxia (FIO_2 = 10%, 4 h) or normoxia (FIO_2 = 21%) exposure. Our data demonstrated that compared with Ringer-infused animals, in hypoxia and normoxia, PBvHb treatment improved oxygen content but raised mean arterial pressure, lowered stroke volume, heart rate, and cardiac index, which resulted in a net reduction in blood flow and oxygen delivery to the tissues. The PBvHb vasoactive effect was similar in magnitude and direction as to the Ringer-infused animals treated with a nitric oxide synthase inhibitor nitro-L-arginine, suggesting the PBvHb effect is mediated via nitric oxide scavenging. We conclude that infusion of PBvHb is not likely to be useful in treating global hypoxia under these conditions.

hemoglobin-based oxygen carriers; Nitric oxide; Cardiac output

HEMOGLOBIN-BASED OXYGEN CARRIERS (HBOCs) were originally designed to improve oxygen carrying capacity and blood volume in patients suffering from acute blood loss (15, 22). HBOCs have the attractive features of universal compatibility, long storage life, and reduction of disease transmission (15). A wide variety of other potential therapies have recently been proposed for HBOCs such as adjunct therapy during radiation and chemotherapy (19), management of pedicled flaps (18), reducing infarct volume as a result of focal cerebral ischemia (16), and scavenging nitric oxide (19).

HBOCs that are of clinical interest currently constitute several types, based on the chemical modification of the hemoglobin molecules to provide longer circulation times and optimal oxygen affinity and decrease negative side effects. These HBOC types have been reviewed recently by Winslow (26) and are categorized into cross-linked, polymerized, and conjugated hemoglobin. Cell-free hemoglobin has long been associated with hypertension when infused (26). Almost all polymerized hemoglobin variants induced variable degrees of vasoconstriction (9, 15, 20). Although the cause is still debated, most HBOC vasoconstrictive properties likely stem from nitric oxide scavenging (17, 23). In contrast, Tsai et al. (23) demonstrated that polyethylene-glycol-decorated polymerized Hb had similar nitric oxide affinity as other polymerized Hb derivatives but did not produce vasoconstriction. Thus, this supports the alternative theories that vasoconstriction may be a result of supraphysiological oxygenation of tissues surrounding blood vessels, dependent on the molecular volume of the oxygen binding sites on HBOCs (26), or due to an oncotic volume expansion effect (14).

HBOC therapies have been primarily studied for blood loss treatment (7, 12, 15, 24), and studies investigating HBOC as a means for enhanced oxygen therapy, other than for blood loss treatment, are few (16, 18). Unfortunately, these studies either focused on the hemodynamics or on the oxygen binding characteristics of HBOC but failed to comprehensively provide data on the influence of HBOC-induced hemodynamic changes on oxygen delivery per se. HBOC infusion in euvolemic individuals would likely occur during radiation and chemotherapy therapy, pedicled flap treatment, and cerebral ischemia to enhance tissue oxygen delivery in such conditions.

We hypothesized that administration of a gluteraldehyde-polymerized bovine hemoglobin (PBvHb) to euvolemic animals during acute global hypoxia would increase the oxygen carrying capacity of blood and oxygen delivery to tissues, despite the possibility of PBvHb-induced vasoconstriction and other hemodynamic changes, such as reduced cardiac output. PBvHb was chosen for this study, as this HBOC has been approved for veterinary use in the United States and South Africa and for limited use in humans in South Africa.

Our primary goal was to determine the oxygen delivery and systemic and pulmonary hemodynamic effects after a 3-ml infusion of PBvHb during acute hypoxia compared with normoxia in a conscious rat model of global hypoxia. Therefore, studies were designed to measure oxygen content, blood gases,

Address for reprint requests and other correspondence: D. Irwin, Univ. of Colorado Health Science Center, Cardiovascular Pulmonary Research Laboratory, School of Medicine, 4200 E 9th Ave., Denver, CO 80262 (e-mail: David.irwin@UCHSC.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Table 1. *Physical characteristics of polymerized bovine hemoglobin*

Physical Characteristic of Polymerized Bovine Hemoglobin Glutamer 200	
Mean molecular mass, kDa	180 (32–500) >95%
Tetramers, %	37
Unstabalized tetramers, %	<5*
Hemoglobin, g/dl	13.1
P_{50}	38
Colliod osmotic potential, mosmol/kgH_2O	290–310
Oncotic pressure, mmHg	17
Viscosity, cP	1.8
Hb saturation room air, %	72
Met Hb, %	<5
Endotoxin units, U/ml	<0.05
pH	7.6–7.9

Colloid osmotic potential was at 27°. metHb, methemoglobin. See Ref. 3 for mean molecular mass and tetramer details. Other information is from Refs. 5 and 10 and from manufacturer's product description.

and pulmonary and systemic hemodynamics in awake, conscious rats exposed to either 21 or 10% oxygen. Secondarily, the study was designed to determine the potential role of nitric oxide in PBvHb-induced vasoactivity.

METHODS

Animals

Male Sprague-Dawley rats (280–350 g and 10–12 wk of age) were obtained from a commercial vendor (Charles River) and housed in the University of Colorado Health Sciences Center's Center for Laboratory Animal Care (elevationn = 5,280 ft). Animals were allowed ad libitum access to food and water and were kept on a 12-h day-night cycle. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at University of Colorado Health Sciences Center.

Instrumentation

Rats were allowed to acclimate to Denver altitude (5,280 ft; 1,690 m; $P_B \sim 630$ mmHg) for at least 7 days before instrumentation. Forty-eight hours before surgery, rats were provided water supplemented with acetaminophen/codeine (0.5 mg/ml and 0.05 mg/ml, respectively) for postoperative analgesia. The animals were weighed, and the hematocrits were determined. Rats were anesthetized with a mixture of ketamine: Rompon (xylazine; 75 mg/kg: 6 mg/kg ip). Under aseptic conditions, the left carotid artery was cannulated with a PE-50 (0.58 mm ID, Becton Dickinson) catheter. A PV-1 (0.28 mm ID, Becton Dickinson) catheter with a shallow bend at its tip was inserted into the right ventricle via the right jugular vein and guided into the main pulmonary artery. Pressure tracings confirmed placement in the pulmonary artery. Next, two PE-50 (0.58 mm ID, Becton Dickinson) catheters were placed in the superior vena cava via the right jugular vein for venous blood collection and to obtain cardiac output values. All catheters were flushed with heparinized saline, tied off, tunneled subcutaneously to the dorsal neck region, and exteriorized at the back of the neck. Animals were allowed at least 48 h to recover before any treatments. Animals demonstrating signs of infection, diarrhea, or distress were excluded from study.

Estimation of Blood Clearance Rate and Physical Characteristics of PBvHb

It has previously been suggested that the PBvHb plasma concentration half-life is species and dose dependent (16, 13). Therefore, the PBvHb clearance rate was estimated in male Sprague-Dawley rats ($n = 4$) infused with 1.3 g/kg PBvHb. Arterial blood was collected hourly for 8 h postinfusion, and plasma PBvHb concentrations were plotted against time to estimate the PBvHb clearance rate in the rat. It was determined that the concentration was reduced by approximately one-half at $\sim 5 \pm 1$ h and undetectable in the blood stream by co-oximetry 24 h after infusion (data not shown). Therefore, in the present study, the PBvHb effects were measured at 2 and 4 h, followed by effects of nitric oxideS inhibition at 4.5 h postinfusion. The physical characteristics of PBvHb "Oxyglobin" are shown in Table 1.

Model and Experimental Design

Animals were randomly assigned into one of four groups, each with a sample size of 10 animals ($n = 10$) unless otherwise specified: *1*) normoxic ($FIO_2 = 21\%$ O_2) lactated Ringer solution (Henery Schein, Melville, NY) infused (Normoxia-R); *2*) normoxic polymerized bovine hemoglobin (Biopure, Cambridge, MA, approved for veterinary use; Table 1) infused (Normoxia-PBvHb); *3*) hypoxic ($FIO_2 = 10\%$) lactated Ringer solution infused (Hypoxia-R); and *4*) hypoxic polymerized bovine hemoglobin infused (Hypoxia-PBvHb).

Hemodynamic Measurements

Rats were placed in a custom designed small, rectangular, plexiglas chambers with a portal through which catheters could be passed. Catheters were flushed with heparinized saline and then connected to

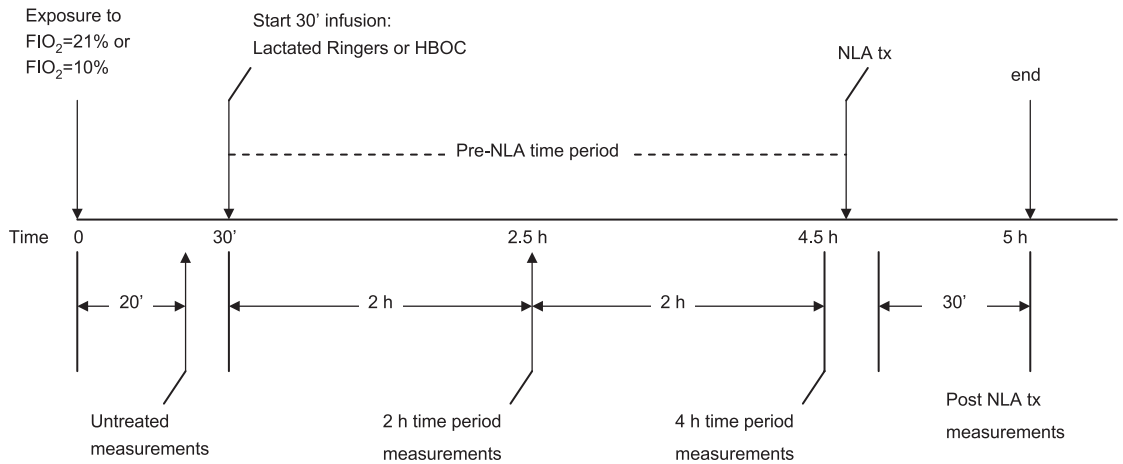

Fig. 1. Experimental protocol. Time points for when lactate Ringer (LR), polymerized bovine hemoglobin, and nitro-L-arginine (NLA) treatments were administered and hemodynamic and blood gas values were recorded. HBOC, hemoglobin-based oxygen carriers.

Table 2. *Blood gas values*

	P_{O_2}, mmHg				P_{CO_2}, mmHg				pH			
	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h
Normoxia-R	76±3	76±2	80±4	77±3	31±1	32±1	30±1	32±1	7.49±0.01	7.51±0.01	7.49±0.02	7.49±0.01
Normoxia-PBvHb	76±3	75±4	80±6	82±6	31±1	31±1	29±2	30±2	7.49±0.01	7.48±0.01	7.52±0.02	7.51±0.01
Hypoxia-R	34±1	34±2	41±2	38±2	22±0.6	22±1	20±1	19±1	7.58±0.01	7.58±0.02	7.58±0.01	7.60±0.01
Hypoxia- PBvHb	34±1	33±1	38±2	39±2	22±0.6	23±1	21±1	19±1	7.58±0.01	7.60±0.01	7.56±0.01	7.59±0.01
	Total Hemoglobin, g/dl				Methemoglobin, % tHb				Hematocrit			
	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h
Normoxia-R												
Blood	14.1±0.5	14.5±0.4	12.7±0.6*	12.3±0.6*	0.1±0.01	0.7±0.21	0.53±0.31	0.40±0.16	44±1	43±1	37±1*	36±1*
Plasma	14.1±0.5	0.0	0.0	0.0	0.1±0.01	0.00	0.00	0.00	44±1			
Normoxia-PBvHb												
Blood	14.1±0.5	13.6±0.7	14.5±0.7*	14.3±0.7*	0.1±0.01	0.16±0.06	0.56±0.17*	0.93±0.35*	44±1	44±1	43±1*	37±1
Plasma	14.1±0.5	0.0	2.2±0.1	1.8±0.07	0.1±0.01	0.00	4.48	11.9±3.5†	44±1			
Hypoxia-R												
Blood	14.1±0.4	14.6±0.4	13.1±0.4	11.4±0.4*	0.05±0.02	0.03±0.01	0.00	0.00	42±1	43±1	40±2	34±1*
Plasma	14.1±0.4	0.0	0.0	0.0	0.05±0.02	0.00	0.00	0.00	42±1			
Hypoxia-PBvHb												
Blood	14.1±0.4	13.5±0.5	15.3±0.3*	15.0±0.2*	0.05±0.02	0.08±0.04	0.32±0.12	0.54±0.18	42±1	41±1	45±2*	38±1*
Plasma	14.1±0.4	0.0	2.3±0.1	2.1±0.1	0.05±0.02	0.00	2.72±0.86	2.57±1.8	42±1			
	Oxygen Saturation, %				Arterial Oxygen Content, vol %				Venous Oxygen Content, vol %			
	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h
Normoxia-R												
Blood	94±1	94±1	96±3	95±1	17.8±0.5	18.4±0.46	15.7±0.59*	15.9±0.66*	10.0±0.75	10.6±0.51	9.23±0.40	9.23±0.14
Plasma	94±1	0.0	0.0	0.0	17.8±0.5	0.0	0.0	0.0	10.0±0.75	0.0	0.0	0.0
Normoxia-PBvHb												
Blood	94±1	94±1	95±1	95±1	17.8±0.5	17.2±0.87	18.4±0.93	18.1±0.84	10.0±0.75	9.4±1.1	9.3±1.0	8.0±0.89
Plasma	94±1	0.0	68±2‡	68±3‡	17.8±0.5	0.0	1.8±0.21	1.4±0.13	10.0±0.75	0.0	1.2±0.04	0.88±0.07
Hypoxia-R												
Blood	52±3	50±6	58±4	61±3	9.9±0.3	9.8±0.62	9.9±0.94	9.5±0.47	6.4±0.34	6.6±0.39	6.8±0.69	5.7±0.71
Plasma	52±3	0.0	0.0	0.0	9.9±0.3	0.0	0.0	0.0	6.4±0.34	0.0	0.0	0.0
Hypoxia-PBvHb												
Blood	52±3	54±1	57±2	63±2	9.9±0.3	9.9±0.31	12.4±0.72*	12.4±6.6*	6.4±0.34	6.2±0.12	7.4±0.95	6.6±0.51
Plasma	52±3	0.0	49.9±2.6‡	58±3‡	9.9±0.3	0.0	1.4±0.13	1.4±0.06	6.4±0.34	0.0	1.3±0.10	1.0±0.07

Values are means ± SE for blood gas values recorded at untreated and 2- and 4-h time points for animals treated with either lacted Ringer or polymerized bovine hemoglobin (PBvHb) in normoxic (FIO_2 =21%, Normoxic-R; Normoxic-PBvHb) or hypoxic (FIO_2 =10%; Hypoxic-R; Hypoxic-PBvHb) conditions. Untreated (whole blood) normoxic or hypoxic means represent all normoxic or hypoxic untreated animals. Untreated means represent group means. *$P < 0.05$ vs. untreated time point; †$P < 0.05$ 2-h v. 4-h time point; ‡$P < 0.05$ vs. whole blood; #$P < 0.05$ vs. Normoxic cohorts.

fluid filled pressure transducers. Animals were exposed to normoxia and hypoxia by flushing the chamber with appropriate gases. Once breathing hypoxic gas, hypoxic animals were not reexposed to room air. Then, blood pressures, pulmonary artery blood pressures, heart rates, and cardiac outputs of normoxic and hypoxic animals were collected before Ringer or PBvHb treatment (Fig. 1).

All animals then underwent a 30 min (10 min per 1 ml) infusion of either Ringer or PBvHb (3 ml/kg, 1.3 g/kg) through a venous catheter. Two and four hours after infusion, hemodynamic variables were measured along with blood gas variables (see below). Then animals were treated with 5 mg/kg of the nitric oxide synthase inhibitor nitro-L-arginine (NLA; Sigma Aldrich, #N10403) administered via a jugular catheter. Hemodynamics were measured 30 min post-NLA infusion. Cardiac output was measured using Cardiogreen (Sigma Aldrich, #I2633) with the dye-dilution method. Between data collection points, the rats were monitored for any signs of distress but were otherwise left undisturbed. Animals were euthanized with an overdose of pentobarbital sodium (100 mg/kg) via a jugular catheter after final measurements.

Blood Gas and Co-Oximetry Measurements for Whole Blood and Plasma

Blood samples were collected immediately after hemodynamic measurements had been obtained at each time point (Fig. 1). Arterial (0.2 ml) and venous (0.2 ml) blood was withdrawn via carotid and venous catheters, respectively, into blood gas syringes and analyzed (ABL5, Radiometer, Copenhagen and co-oximetry via OSM3, Radiometer, Copenhagen) with algorithms specific to rat and bovine hemoglobin.

Plasma phase co-oximetry. Heparinized microhematocrit capillary tubes (100 μl; Fisherbrand, #22362566) were immediately filled from each 1-ml blood gas syringe (see above), sealed from room air with a removable cap (Kendall Healthcare, #8889212000), and spun on a capillary centrifuge to separate cell and plasma fractions. The plasma portion of the capillary tube was aspirated into the co-oximeter. Measurements were excluded if any portion of the cell fraction was inadvertently aspirated into the analyzer. Care was taken to avoid exposing blood samples to room air.

Calculation for Oxygen Delivery, Consumption, and Extraction

Oxygen delivery (DO_2), consumption ($\dot{V}O_2$), and extraction ratio (ERO_2) were calculated for hemoglobin in whole blood and PBvHb in the plasma phase (no measurable endogenous hemoglobin was present in the plasma) with the following mathematical calculations: *1*) $DO_2 = (CaO_2) \times CI$, where CaO_2 and $CvO_2 = (HbO_2\%/100) \times tHb \times \gamma^*$ and $HbO_2 = HbO_2SAT(100 - HbCO - MetHb)$ fraction*; *2*) $\dot{V}O_2 = [(CaO_2) - (CvO_2)] \times CI$; and *3*) $ERO_2 = \dot{V}O_2/DO_2$, where CI is cardiac index and CaO_2 and CvO_2 are arterial and venous content respectively. γ is oxygen capacity for rat or bovine hemoglobin. Asterisks indicate OSM3 programmed algorithms.

Statistics

For all groups, means ± SE are reported. Statistical comparison between groups were analyzed with either a multifactorial (FIO_2, PBvHb-treatment) with repeated measures (Time) ANOVA (see Tables 1, 3–4) or a multifactorial (FIO_2, PBvHb-treatment, NLA-treatment) ANOVA (see Figs. 3–5; Table 2). Unless otherwise noted, the 2- and 4-h time points were combined for determination of either a PBVHB or NLA treatment effect for all hemodynamic values. Post hoc analyses were completed with unpaired, two-sided Student's *t*-test with a Bonferroni adjustment. Statistical analyses were performed using JMP (Version 5) statistical software package (SAS; Cary, North Carolina) with statistical significance set at $P \leq 0.05$.

RESULTS

Total Hemoglobin and Methemoglobin

Normoxia. As expected, the total Hb concentration in whole blood was lower after a 3-ml infusion of Ringer solution due to dilution ($P < 0.01$; Table 2). On the other hand, addition of 3 ml of PBvHb increased the total Hb concentration ~10% ($P < 0.02$; Table 1), due to the presence of PBvHb in the plasma (~2 g/dl; Table 2). An elevation of methemoglobin concentration accompanied the rise in total Hb concentration in whole blood and plasma ($P < 0.04$; Table 1). Remarkably, the methemoglobin concentration in plasma increased approximately sixfold between 2 and 4 h in PBvHb-treated animals ($P = 0.03$, 2 vs. 4 h; Table 2).

Hypoxia. Hypoxia (4 h) per se had no effect on either total or plasma Hb concentration. However, total methemoglobin concentration ($P < 0.001$; Table 1) was lower during hypoxia compared with normoxia. This observation held true for the plasma methemoglobin concentration ($P = 0.04$) of PBvHb-treated animals as well (Table 2). Although plasma methemoglobin concentration increased in PBvHb-treated animals between 2 and 4 h during normoxia, no change between these time points was noted during hypoxia. (Table 2).

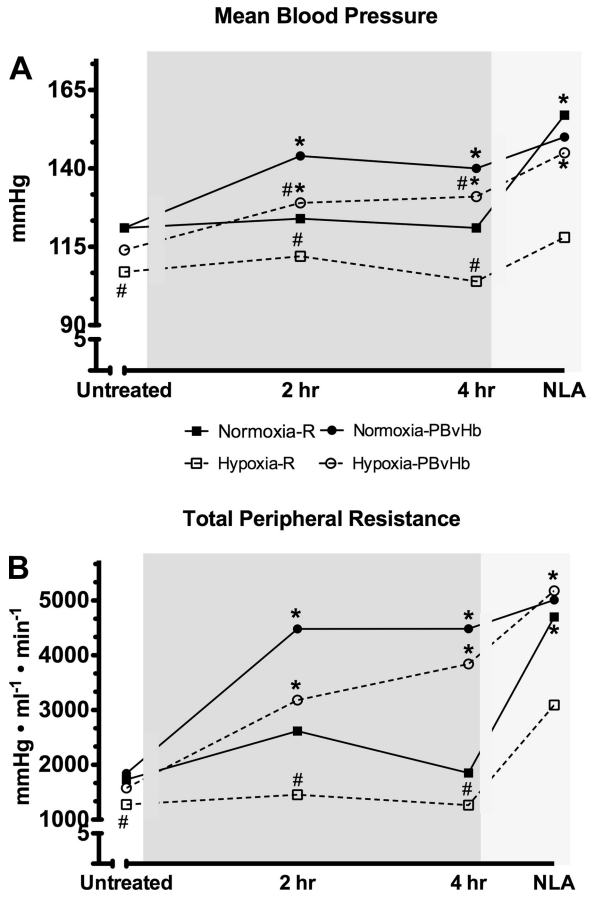

Fig. 2. Systemic blood pressure measurements. *A*: mean arterial pressure. *B*: total peripheral resistance. Data are mean values over the study time course in normoxic ($FIO_2 = 21\%$) or hypoxic ($FIO_2 = 10\%$). *$P < 0.05$ vs. untreated time point; #$P < 0.05$ vs. normoxic cohorts.

Blood Gases

Arterial P_{O_2}, P_{CO_2}, and pH. Neither Ringer nor PBvHb altered P_{O_2}, PCO_2, or pH during normoxia or hypoxia. However, hypoxia produced expected decreases in P_{O_2} and P_{CO_2} and increases in pH ($P < 0.001$; Table 2).

Oxygen Content and Saturation

Normoxia. Whole blood oxygen content was lower after dilution by Ringer infusion ($P = 0.01$; Table 2). Treatment with PBvHb did not improve whole blood oxygen content, yet oxygen content was measurable in the plasma phase (~1.5 mmol; Table 2). Whole blood oxygen saturation was unaffected by either Ringer or PBvHb treatment. In PBvHb-treated animals, oxygen saturation of the plasma phase was lower than oxygen saturation in the whole blood ($P < 0.001$; Table 2).

Hypoxia. As expected, hypoxia decreased oxygen content and saturation in whole blood and plasma ($P < 0.001$; Table 2) before treatment. PBvHb-treated animals had significantly greater whole blood oxygen content within 2 h of hypoxia ($P = 0.005$; Table 2).

Hemodynamics

Unless otherwise stated Ringer infusion had no effect on hemodynamic variables in animals exposed to either normoxia or hypoxia.

Mean arterial pressure and total peripheral resistance. NORMOXIA. The mean arterial pressure (MAP) and total peripheral resistance (TPR) were elevated from untreated values by PBvHb treatment ($P < 0.001$; Fig. 2, *A* and *B*; Table 3). The NLA-induced increase in pressure and resistance in Ringer-infused animals was equivalent to the elevation in MAP and TPR caused by PBvHb alone ($P < 0.001$; Fig. 2, *A* and *B*; Table 4).

HYPOXIA. Compared with normoxia, exposure to acute hypoxia decreased MAP and TPR ($P < 0.001$; Fig. 2, *A* and *B*; Table 3). Treatment with PBvHb then increased MAP and TPR from untreated values by approximately the same magnitude as that observed in animals that had remained in normoxic conditions ($P < 0.001$; Fig. 2, *A* and *B*; Table 3). In the Ringer-infused animals, NLA increased only TPR ($P = 0.01$; Fig. 2*B*; Table 4). NLA treatment further increased both MAP and TPR in the PBvHb infused animals ($P = 0.02$; Fig. 2, *A* and *B*; Table 4).

Table 3. *Hemodynamic values*

	Mean Arterial Pressure, mmHg				Total Peripheral Resistance, mmHg·ml^{-1}·min			
	Untreated Normoxia/Hypoxia	Untreated	2 h	4 h	Untreated Normoxia/Hypoxia	Untreated	2 h	4 h
Normoxia-R	121±4	121±4	124±3	121±4	1,787±180	1,732±178	2,618±417	1,851±190
Normoxia-PBvHb	121±4	121±4	144±5*	140±5*		1,842±182	4,483±586*	4,484±586*
Hypoxia-R	111±3	107±3	112±4	104±6	1,425±122	1,274±144	1,455±193	1,263±220
Hypoxia-PBvHb	111±3	114±3	129±4*	131±4*		1,576±126	3,181±532*	3,843±673*
	Mean Pulmonary Arterial Pressure, mmHg				Total Pulmonary Resistance, mmHg·$^{-1}$·min			
	Untreated Normoxia/Hypoxia	Untreated	2 h	4 h	Untreated Normoxia/Hypoxia	Untreated	2 h	4 h
Normoxia-R	19±1	19±1	16±1	17±1	325±46	279±35	323±74	264±29
Normoxia-PBvHb	19±1	20±2	23±2	22±2†		371±57	757±144*	690±116*
Hypoxia-R	25±1	25±1	26±1	26±1	288±21	269±24	407±57	381±64
Hypoxia-PBvHb	25±1	25±1	34±1*	30±2*		308±18	775±77*	789±83*
	Cardiac Index, ml·min^{-1}·kg^{-1}				Stroke volume, ml/kg			
	Untreated Normoxia/Hypoxia	Untreated	2 h	4 h	Untreated Normoxia/Hypoxia	Untreated	2 h	4 h
Normoxia-R	219±20	214±19	180±42	198±17	0.55±0.04	0.55±0.04	0.54±0.11	0.53±0.03
Normoxia-PBvHb	219±20	224±20	108±12*	124±14*		0.55±0.06	0.35±0.05*	0.34±0.05*
Hypoxia-R	252±18	262±16	236±29	255±27	0.58±0.05	0.59±0.08	0.57±0.08	0.61±0.06
Hypoxia-PBvHb	252±18	242±20	149±14*	130±12*		0.56±0.06	0.39±0.04*	0.33±0.03*
	Heart Rate, beats/min				Pulse Pressures, mmHg			
	Untreated Normoxia/Hypoxia	Untreated	2 h	4 h	Untreated Normoxia/Hypoxia	Untreated	2 h	4 h
Normoxia-R	401±10	408±9	368±31*	392±9	30±2	27±2	29±2	34±5
Normoxia-PBvHb	401±10	394±11	314±16*	373±16		34±2	30±4	27±3*
Hypoxia-R	433±18	434±19	424±15	431±12	38±3	38±4	37±4	40±8
Hypoxi-PBvHb	433±18	432±17	380±15*	383±18*		39±3	29±2*	29±2*

Values are means ± SE for hemodynamic values recorded at untreated and 2- and 4-h time points for animals treated with either lacted Ringer or PBvHb in normoxic ($FIO_2 = 21\%$; Normoxia-R; Normoxia-PBvHb) or hypoxic ($FIO_2 = 10\%$; Hypoxia-R; Hypoxia-PBvHb) conditions. *$P < 0.05$ vs. untreated time point, †$P < 0.05$ vs. Normoxia cohorts, ‡$P < 0.05$ vs. Ringer cohorts.

Table 4. *NOS inhibition effect*

	Mean Arterial Pressure, mmHg		Total Peripheral Resistance, mmHg·ml^{-1}·min	
	Pre-NLA	NLA*	Pre-NLA	NLA*
Normoxia-R	122±4.7	157±14*	2,234±422	4,701±316*
Normoxia-PBvHb	142±3.8	150±12	4,345±336	5,009±295
Hypoxia-R	108±4.8	118±6.1	1,358±309	3,093±306
Hypoxia-PBvHb	130±3.5	145±6.8*	3,512±309	5,179±277*
	Pulmonary Arterial Pressure, mmHg		Total Pulmonary Resistance, mmHg·ml^{-1}·min	
	Pre-NLA	NLA*	Pre-NLA	NLA*
Normoxia-R	17±3	20±3	441±72	652±75*
Normoxia-PBvHb	22±2	20±4	724±108	470±72
Hypoxia-R	26±1	31±2*	393±127	859±183*
Hypoxia-PBvHb	32±1	26±3	782±100	949±92
	Cardiac Index, ml·min^{-1}·kg^{-1}		Stroke Volume, ml/beat	
	Pre-NLA	NLA*	Pre-NLA	NLA*
Normoxia-R	188±20	93±13*	0.53±0.05	0.27±0.04*
Normoxia-PBvHb	116±18	106±9	0.34±0.02	0.35±0.05
Hypoxia-R	245±22	126±16*	0.59±0.03	0.37±0.05*
Hypoxia-PBvHb	140±17	101±12*	0.36±0.02	0.19±0.03*
	Heart Rate, beats/min		Pulse Pressures, mmHg	
	Pre-NLA	NLA*	Pre-NLA	NLA*
Normoxia-R	380±23	313±20*	31±3	22±3*
Normoxia-PBvHb	343±17	354±30	28±2	22±4
Hypoxia-R	427±15	378±19*	39±3	24±3*
Hypoxia-PBvHb	381±11	346±21	30±2	23±4*

Values are means ± SE for the hemodynamic values recorded before and after treatment with the NOS inhibitor NLA for animals treated with either lacted Ringer or PBvHb in normoxic (FIO_2 = 21%; Normoxia-R; Normoxia-PBvHb) or hypoxic (FIO_2 = 10%; Hypoxia-R; Hypoxia-PBvHb) conditions. PreNLA data values are the means ± SE of the 2- and 4-h time points pooled. ‡$P < 0.05$ vs. preNLA values; *$n = 6$ per group. Note: pre NLA group is combined data from 2- and 4-h time points.

Mean pulmonary artery pressure and total pulmonary resistance. NORMOXIA. We observed no change from untreated values in mean pulmonary artery pressures in animals treated with PBvHb, but a significant increase in total pulmonary vascular resistance was noted ($P < 0.001$; Fig. 3, *A* and *B*; Table 3). Treatment with NLA had no effect on mean pulmonary artery pressures in either the Ringer or PBvHb groups (Fig. 3, *A* and *B*; Table 4). However, NLA increased pulmonary vascular resistance in the Ringer-infused group to a similar extent as that observed after PBvHb treatment ($P = 0.04$; Fig. 3*B*; Table 4).

HYPOXIA. As expected, mean pulmonary artery pressure was increased with hypoxia exposure (Table 3). The initial increase in pulmonary artery pressure induced solely by hypoxia was further increased with PBvHb treatment, along with an increase in pulmonary vascular resistance ($P < 0.001$; Fig. 3, *A* and *B*; Table 3). The pulmonary artery pressure and pulmonary vascular resistance were increased further by NLA in the Ringer-infused animals ($P = 0.03$; Fig. 3, *A* and *B*; Table 4). However, NLA had no additional influence on the PBvHb-induced increase in pulmonary hemodynamics (Fig. 3, *A* and *B*).

Heart rate and pulse pressures. NORMOXIA. Infusion of Ringer or PBvHb solution caused a brief but transient decrease in heart rate that was resolved by 4 h ($P = 0.02$ for Ringer and $P = 0.003$ for PBvHb; Fig. 4*A*; Table 3). However, pulse pressure decreased from untreated values after PBvHb but not with Ringer treatment ($P < 0.001$; Table 1). NLA decreased HR and pulse pressure only in the Ringer-infused animals ($P = 0.04$ for HR; Fig. 4*A*; and $P < 0.001$ for pulse pressure; Table 4).

HYPOXIA. Hypoxia caused an increased HR that was reversed by treatment with PBvHb ($P = 0.04$; Fig. 4*A*; Table 3). Hypoxia did not alter pulse pressure in Ringer-infused animals, while pulse pressure was lowered by PBvHb treatment. ($P < 0.001$; Table 2). Treatment with NLA decreased resting HR and pulse pressure in the Ringer-infused animals ($P < 0.006$; Fig. 4*A*; Table 4) but had no effect on the PBvHb-treated animals (Fig. 4*A*; Table 4).

Cardiac index and stroke volume. normoxia. Treatment with PBvHb induced a dramatic decrease in cardiac index and stroke volume ($P \leq 0.002$; Fig. 4, *B* and *C*; Table 3). NLA decreased cardiac index and stroke volume in Ringer-infused animals ($P \leq 0.02$; Fig. 4, *B* and *C*; Table 4), but did not change cardiac index and stroke volume in the PBvHb-treated group.

HYPOXIA. Hypoxic stress increased the cardiac index (Table 3). However, hypoxia PBvHb treatment greatly decreased cardiac index and stroke volume, as had occurred in normoxia

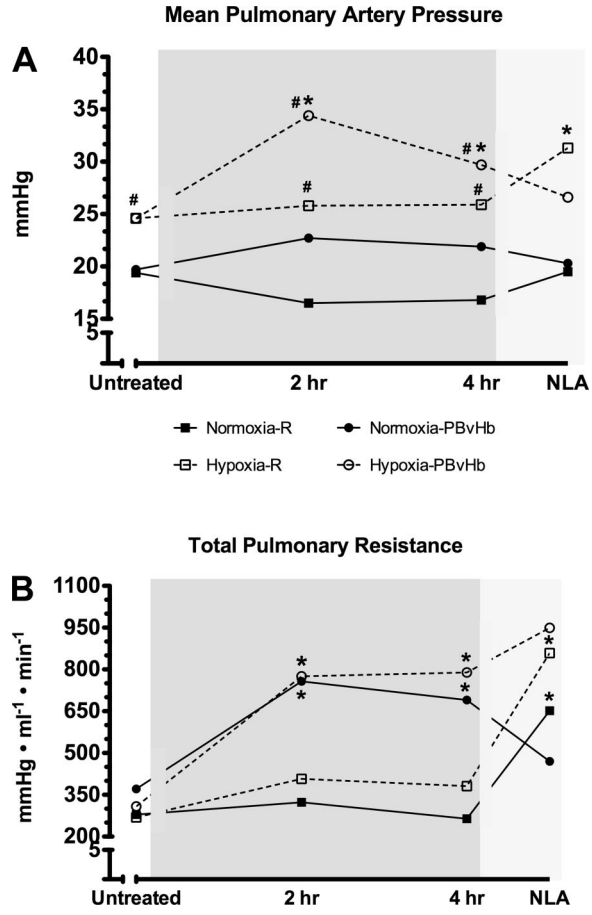

Fig. 3. Pulmonary blood pressure measurements. *A*: mean pulmonary arterial pressure. *B*: total pulmonary vascular resistance. Data are mean values over the study time course in normoxic (FIO_2 = 21%) or hypoxic (FIO_2 = 10%). *$P <$ 0.05 vs. untreated time point; #$P < 0.05$ vs. normoxic cohorts.

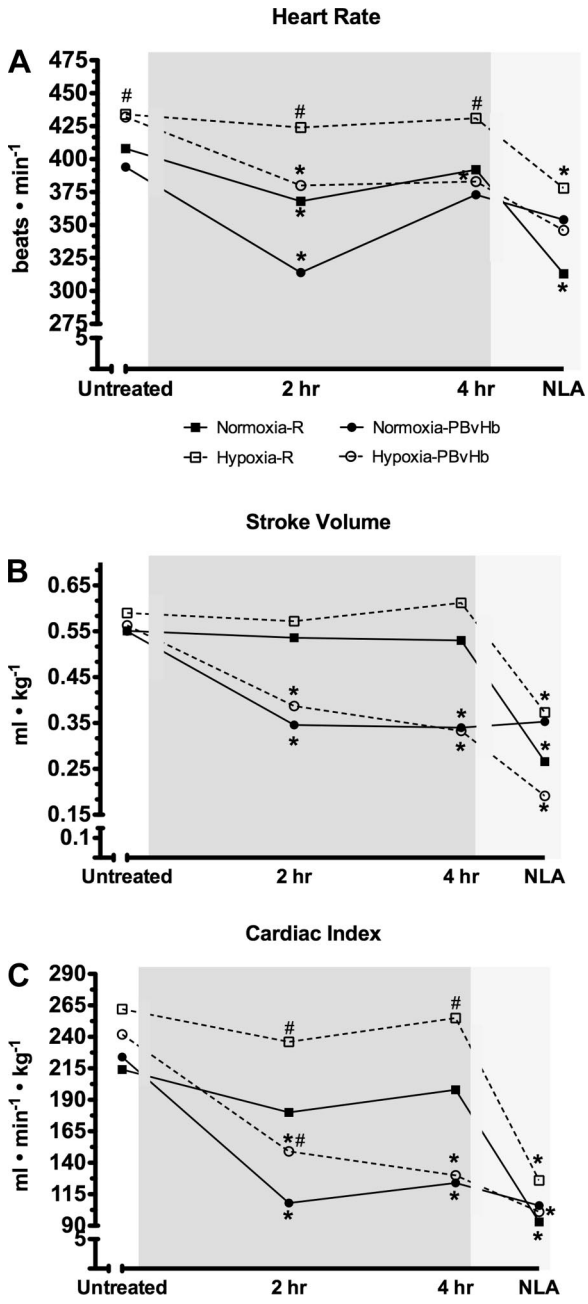

Fig. 4. Cardiac function: *A*: heart rate. *B*: stroke volume. *C*: cardiac index. Data are mean values over the study time course in normoxic (FIO_2 = 21%) or hypoxic (FIO_2 = 10%). *$P < 0.05$ vs. untreated time point; #$P < 0.05$ vs. normoxic cohorts.

($P = 0.006$; Fig. 4, *B* and *C*; Table 3). In Ringer-infused animals, treatment with NLA induced a decrease in cardiac index and stroke volume similar in magnitude to that observed in PBvHb-treated animals ($P \leq 0.02$; Fig. 4, *B* and *C*; Table 4). Interestingly, NLA further decreased cardiac index and stroke volume in the PBvHb animals ($P < 0.001$; Fig. 4, *B* and *C*; Table 4).

Oxygen delivery, Consumption, and Extraction

Normoxia. Treatment with PBvHb reduced oxygen delivery and $\dot{V}O_2$ ($P < 0.001$; Fig. 5; Table 5), but Ringer infusion did not. Thus the calculated extraction rate for oxygen was less with PBvHb compared with Ringer treatment during normoxia. The reduction in oxygen extraction from whole blood was greater than that from plasma ($P < 0.001$; Table 5).

Hypoxia. Hypoxia induced a reduction in oxygen delivery in all animals compared with normoxia, while PBvHb treatment further decreased oxygen delivery ($P < 0.001$; Fig. 5; Table 5) during hypoxia. However, the magnitude of the PBvHb-induced reduction in oxygen delivery was not as great as that recorded during normoxia. Initially, exposure to hypoxia lowered $\dot{V}O_2$ in all animals ($P < 0.001$; Table 5). However, in Ringer-infused animals $\dot{V}O_2$ had returned to normoxic values by 4 h, while PBvHb treatment during hypoxia lowered $\dot{V}O_2$ even below that in normoxic PBvHb animals.

In all animals, hypoxia induced a rise (~18%) in whole blood oxygen extraction ratio over the time course of the study period ($P = 0.038$, untreated vs. 2 and 4 h pooled; Table 4). However, in PBvHb-treated animals, the oxygen extraction ratio for plasma was lower during acute hypoxia than during normoxia but doubled between the 2- and 4-h time periods ($P = 0.02$; Table 4).

DISCUSSION

The goal of this study was to determine if an infusion of an HBOC (PBvHb) could be used to improve oxygen delivery during hypoxia in a conscious euvolemic animal and, thus, be efficacious for use during radiation and/or chemotherapy and clinical conditions of euvolemic hypoxic stress. The data do not support the use of PBvHb for such conditions. PBvHb therapy had a far greater negative impact on cardiac index than was previously recognized in reports using anesthetized animals. Thus although PBvHb treatment slightly improved blood oxygen content, the reduction in cardiac index resulted in a large net decrease in oxygen delivery to the tissues during both normoxia and hypoxia. As expected, nitric oxide scavenging played a significant role in the hemodynamically mediated reduction in oxygen delivery changes with PBvHb treatment. Therefore, HBOCs that severely compromise cardiac index and blood flow are not likely to be useful in resolving tissue hypoxia experienced during radiation, chemotherapy, pedicled

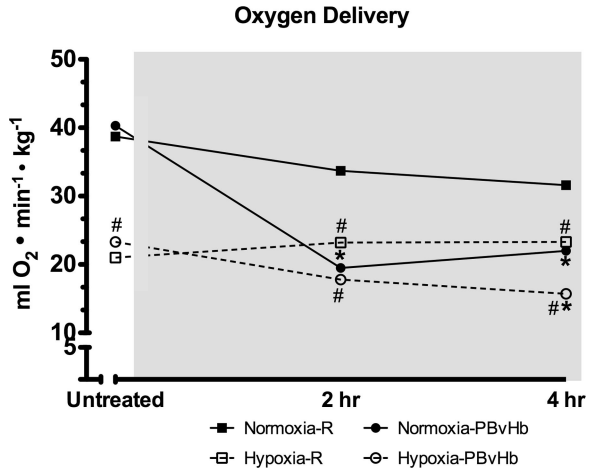

Fig. 5. Calculated oxygen delivery. Data are mean values over the study time course in normoxic (FIO_2 = 21%) or hypoxic (FIO_2 = 10%). *$P < 0.05$ vs. untreated time point; #$P < 0.05$ vs. normoxic cohorts.

Table 5. *Oxygen delivery, extraction ratio, and consumption values*

	Oxygen Delivery, ml·O_2 min^{-1}·kg^{-1}			
	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h
Normoxia-R				
Blood	39±3	39±3	34±11	32±3
Plasma	39±3	NA	NA	NA
Normoxia-PBvHb				
Blood	39±3	40±5	20±2*	22±3*
Plasma	39±3	NA	1.8±0.3	1.7±0.2
Hypoxia-R				
Blood	22±3	21±4	23±3	23±3
Plasma	22±3	NA	NA	NA
Hypoxia-PBvHb				
Blood	22±3	23±2	18±2	16±2*
Plasma	22±3	NA	2.3±0.4	1.8±0.3
	Oxygen Extraction Ratio			
	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h
Normoxia-R				
Blood	44±4	43±3	41±6	38±5
Plasma	44±4	0.0	0.0	0.0
Normoxia-PBvHb				
Blood	44±4	46±6	46±5	52±4
Plasma	44±4	0	28±6‡	33±6‡
Hypoxia-R				
Blood	38±3	38±4	43±3	51±5*
Plasma	38±3	0	0	0
Hypoxia-PBvHb				
Blood	38±3	40±3	47±3*	43±3
Plasma	38±3	0.0	10±4‡	21±3†‡
	Oxygen Consumption, ml·O_2 min^{-1}·kg^{-1}			
	Untreated (blood) Normoxia/Hypoxia	Untreated	2 h	4 h
Normoxia-R				
Blood	16±3	14±2	12±4	13±1
Plasma	16±3	NA	NA	NA
Normoxia-PBvHb				
Blood	16±3	17±4	9±1*	11±1*
Plasma	16±3	NA	0.42±0.06	0.64±0.07
Hypoxia-R				
Blood	9±2	9±3	10±2	12±2
Plasma	9±2	NA	NA	NA
Hypoxia -PBvHb				
Blood	9±2	10±2	7±2	6±0.5*
Plasma	9±2	NA	0.31±0.12	0.40±0.11

Values are means ± SE for values recorded at untreated and 2- and 4-h time points for animals treated with either lacted Ringer or PBvHb in normoxic (FIO_2 = 21%, Normoxic-R; Normoxic-PBvHb) or hypoxic (FIO_2 = 10%; Hypoxic-R; Hypoxic-PBvHb) conditions. Blood, whole blood. *$P < 0.05$ vs. untreated time point; †$P < 0.05$ 2 h vs. 4 h time point; ‡$P < 0.05$ vs. whole blood; #$P < 0.05$ vs. Normoxic cohorts.

flap, and cerebral ischemia treatment, unless the hemodynamic side effects can be reduced and/or ameliorated.

The HBOC used in this study consisted of purified gluteraldehyde cross-linked PBvHb. The species differences, purity, and/or specific cross-link may have contributed to some of the adverse effects seen in this rat model. For example, compared with other products, HBOCs prepared from human hemoglobin cross-linked with pyridoxal-5′-phosphate followed with polyoxyethylene glycol have been shown to be less vasoactive in exchange transfusion models (1, 2).

The time course of the experiments and the infused concentration of PBvHb were selected based on estimated HBOC clearance rate (see METHODS) and the in situ concentration of PBvHb necessary to increase the hypoxic blood oxygen content to that of the normoxic oxygen content levels. Clearance rates in the current study are similar to those previously published for mice (16). The volume (3 ml) of PBvHb or Ringer solution infused over a 30-min period did not cause hemodynamic changes and could be safely infused without adverse effects due to volume overload, as has been previously reported (21). The PBvHb dose (1.3 g/kg) was one- to threefold higher than what others have used in hypervolemic models (6, 23). However, the goal of these previous studies (6, 23) was to study the reaction between various HBOC and nitric oxide and not to increase oxygen content to compensate for hypoxia. Interestingly, Tsai et al. (23) suggested that the microvascular effects of HBOC may not be based on concentration but on HBOC properties such as molecular modification, molecular volume, and oxygen affinity. In fact, data indicate that as little as 0.5 g/dl of HBOC is sufficient to scavenge most intravascular nitric oxide (23). Therefore, the larger dose of PBvHb used in the current study to compensate for a hypoxia-induced reduction in arterial oxygen content should not have had an additional nitric oxide scavenging effect.

Blood Gas Variables

A primary goal of this study was to determine whether or not PBvHb infusion could enhance blood oxygen content sufficiently enough to compensate for low arterial oxygen saturation and content during acute hypoxia. As expected PBvHb infusion increased total hemoglobin and arterial oxygen concentration in whole blood by ~10% in all animals. The difference in whole blood and plasma hemoglobin oxygen saturation from the current study is in agreement with previous findings in which cross-linked PBvHb have shown altered oxygen binding characteristics (3). However, our data showed that increased total hemoglobin and oxygen content tracked together at ~10% after PBvHb infusion. This would suggest that the HBOC oxygen binding was sufficient to maintain an equal relationship between total hemoglobin and oxygen content. Thus although PBvHb has different oxygen binding characteristics than native hemoglobin, PBvHb infusion did increase oxygen content during hypoxia sufficiently to offset the loss of oxygen content that occurred during hypoxia exposure.

HBOC Hemodynamic Effects During Normoxia

Because oxygen delivery is determined by oxygen content and cardiac output, the finding that oxygen content was improved with PBvHb but cardiac output was markedly decreased in normoxia is of importance to therapeutic considerations. This is the first study to report such a large fall in cardiac output with PBvHb infusion during normoxia. The finding that PBvHb infusion increased MAP and TPR in normoxic conditions is in agreement with other studies (6, 14, 17, 23) demonstrating an PBvHb-induced systemic pressor response. It is likely that greater MAP and peripheral resistance increased ventricular afterload, decreased heart rate and stroke volume, and thus impaired cardiac output (8).

Because inhibition of nitric oxide synthase in normoxia did not cause a further rise in systemic resistance or cardiac output, it is likely that nitric oxide scavenging by PBvHb was ulti-

mately responsible for the reduction in cardiac output. It is also possible that PBvHb had a direct effect on cardiac function by inducing cardiac lesions, though this has not been demonstrated in the rat, compared with other species (4). However, investigators (4) did not demonstrate that the heart lesions directly decreased cardiac index. Overall, it appears that during normoxia, nitric oxide scavenging by PBvHb increased left ventricular afterload, lowering cardiac index.

PBvHb Hemodynamic Effects During Acute Hypoxia (FIO_2 = 10%)

During hypoxia, PBvHb caused a further reduction in oxygen delivery despite the maintenance of oxygen content. This was directly related to a PBvHb-induced decrease in cardiac output similar to that observed in normoxia. However, our data suggest that in hypoxic conditions PBvHb further elevated mean pulmonary artery pressure and subsequently increased right ventricular afterload, likely contributing to the lowering of cardiac output. This reduction in cardiac output occurred in combination with the elevated MAP and left ventricular afterload.

Nitric oxide synthase inhibition further elevated pulmonary artery pressure during hypoxia in Ringer-infused animals but not PBvHb-treated animals. Thus the PBvHb-induced decrease of cardiac output is likely indirectly mediated by vascular nitric oxide scavenging by HBOC during both normoxia and hypoxia; however, the lower cardiac output appears to be primarily due to elevated right ventricular afterload during hypoxia compared with increased left ventricular afterload during normoxia.

An interesting observation regarding hypoxia and systemic pressures requires addressing. Although hypoxia generally increases systemic pressures, the reduction in peripheral vascular resistance and blood pressures in all animals during hypoxia in the present study may be explained by earlier reports in which acute hypoxic insult resulting in arterial $P_{O_2} < 40$ mmHg (as in the current study) caused locally mediated vasodilation and, thus, decreased arterial blood pressure (11, 25). Although PBvHb treatment (and nitric oxide scavenging) during hypoxia elevated vascular resistance and blood pressure, pressures were not returned to normoxic values after PBvHb treatment. This suggests that the mechanism of hypoxia-induced systemic vasodilation in the present study was not completely nitric oxide mediated. Interestingly, treatment with a nitric oxide synthase inhibitor during hypoxia further elevated blood pressure and vascular resistance in PBvHb but not Ringer-treated animals. There is no clear cause of this phenomenon, and more research is necessary to elucidate this issue.

Mechanisms of PBvHb-Induced Vasoconstriction

Nitric oxide scavenging by HBOC is a primary contributor to rising systemic pressure during normoxia (1, 2, 6, 14, 17, 23), and our data confirm that it also contributes significantly to PBvHb-induced elevation of pulmonary artery pressure during hypoxia in conscious animals. However, PBvHb-induced vasoconstriction could also be influenced by volume expansion due to elevated intravascular oncotic pressure (12) and/or an "autoregulatory" vasoconstrictive effect in which a high P_{50} Hb favoring rapid oxygen unloading exposing arteriolar vessel walls to supranormal PO_2, triggering vasoconstriction (26).

Oxygen Delivery, Consumption, and Extraction

PBvHb impaired oxygen delivery during both normoxia and hypoxia. The 50% reduction in cardiac index with PBvHb infusion accounts for the decreased oxygen delivery as discussed above. Interestingly, during both normoxia and hypoxia, PBvHb decreased oxygen consumption significantly. This is notable, because unless aerobic metabolism is decreased, one would expect oxygen consumption to be maintained near baseline values throughout the 4-h study period. Interestingly, Cabrales (5) observed a similar response in the microvasculature in a Hamster window chamber experiment 1 h after an 80% exchange transfusion. Similar to those experiments, it is hard for us to draw any conclusion as to whether or not this is a positive or negative consequence of PBvHb. Perhaps PBvHb treatment and its vascular effects resulted in the preferential diversion of blood flow from higher to lower oxygen consuming tissue, but this will require further investigation.

Oxygen extraction, a function of oxygen delivery vs. oxygen consumption, was greater in whole blood than in plasma during PBvHb treatment under normoxic and hypoxic conditions. This is in agreement with previous studies of oxygen transport during PBvHb treatment (24). During hypoxia in Ringer-infused animals, as expected, the extraction of oxygen by the tissues (as reflected by the extraction ratios) increased over the 4-h study period to compensate for decreased oxygen delivery, maintaining oxygen consumption. In contrast, with PBvHb treatment during normoxia and hypoxia, the extraction of oxygen by the tissue did not increase sufficiently enough to maintain oxygen consumption. This important observation requires further investigation to determine how PBvHb may reduce oxygen consumption and extraction.

In conclusion, hemodynamic and blood gas data indicate that PBvHb infusion was ineffective as a means for increasing oxygen delivery during acute hypoxic insult. In fact, PBvHb had a negative consequence on oxygen delivery during both normoxia and hypoxia. The primary mechanism for the negative effects of PBvHb during isovolemic hypoxia appears to be nitric oxide scavenging with confounding effects on cardiac output, oxygen extraction, and oxygen consumption. It remains to be determined if eliminating the nitric oxide scavenging properties of PBvHb will enable PBvHb to become an effective oxygen carrier and oxygen deliverer under normoxic and/or hypoxic conditions.

ACKNOWLEDGMENTS

We thank Dr. Oka Masakahiko for expertise in NLA treatment and Dr. Susan Kayar for providing scientific expertise throughout the project.

GRANTS

This work was supported by the Defense Advanced Research Projects Agency and the U.S. Army Research Office (contract number W911NF-06-1-0318) and in part by National Institutes of Health National Research Service Award Training Grant (to D. C. Irwin).

REFERENCES

1. **Alayash AI.** Hemoglobin-based blood substitutes: oxygen carriers, pressor agents, or oxidants? *Nat Biotechnol* 17: 545–549, 1999.
2. **Alayash AI.** Oxygen therapeutics: can we tame haemoglobin? *Nat Rev Drug Discovery* 3: 152–159, 2004.
3. **Buehler PW, Boykins RA, Jia Y, Norris S, Freedberg DI, Alayash AI.** Structual and functional characterization of glutaraldehyde-polymerized

bovine hemoglobin and its isolated fractions. *Anal Chem* 77: 3466–3478, 2005.
4. **Burhop K, Gordon D, Estep T.** Review of hemoglobin-induced myocardial lesions. *Artif Cells Blood Substit Immobil Biotechnol* 32: 353–374, 2004.
5. **Cabrales P, Tsai AG, Intaglietta M.** Increased tissue P_{O_2} and decreased O_2 delivery and consumption after 80% exchange transfusion with polymerized hemoglobin. *Am J Physiol Heart Circ Physiol* 287: H2825–H2833, 2004.
6. **Doherty DH, Doyle MP, Curry SR, Vali RJ, Fattor TJ, Olson JS, Lemon DD.** Rate of reaction with nitric oxide determines the hypertensive effect of cell-free hemoglobin. *Nat Biotechnol* 16: 672–676, 1998.
7. **Glasgow SC, Shah AS, Noone RB, Gottfried MR, Eachempati SR, Talarico TL, Vaslef SN.** Comparison of various hemoglobin polyoxethylene conjugate solutions as resuscitative fluids after hemorragic shock. *J Trauma* 48: 884–893, 2000.
8. **Guyton A, Jones C, Coleman T.** *Circulatory Physiology: Cardiac Output and Its Regulation*. Philidelphia, PA: WB Saunders, 1973.
9. **Hess JR, MacDonald VW, Brinkley WW.** Systemic and pulmonary hypertension after resuscitation with cell-free hemoglobin. *J Appl Physiol* 74: 1769–1778, 1993.
10. **Hughes GS, Francome SF, Antal EJ, Adams WJ, Locker PK, Yancey EP, Jacobs EE Jr.** Hematologic effects of a novel hemoglobin-based oxygen carrierr in normal male and femal subjects. *J Lab Clin Med* 126: 444–451, 1995.
11. **Hultgren H.** *High Altitude Medicine*. Stanford, CA: Hultgren, 1997, p. 520.
12. **Jahr JS, Lurie F, Driessen B, Tang Z, Lourie RF, Kost G.** Validation of oxygen saturation measurements in a canine model of hemoglobin-based oxygen carrier infusion. *Am J Ther* 10: 21–28, 2003.
13. **Lee R, Neya K, Svizzero TA, Vlahakes GJ.** Limitations of the efficacy of hemoglobin-based oxygen-carrying solutions. *J Appl Physiol* 79: 236–242, 1995.
14. **Migita R, Gonzales A, Gonzales ML, Vandegriff KD, Winslow RM.** Blood volume and cardiac index in rats after exchange transfusion with hemoglobin-based oxygen carriers. *J Appl Physiol* 82: 1995–2002, 1997.
15. **Moore EE, Johnson JL, Cheng AM, Masuno T, Banerjee A.** Insights from the studies of blood substitutes in trauma. *Shock* 24: 197–205, 2005.
16. **Nemoto M, Mito T, Brinigar W, Fronticelli C, Koehler RC.** Salvage of focal cerebral ischemic damage by transfusion of high O2-affinity recombinant hemoglobin polymers in mouse. *J Appl Physiol* 100: 1688–1691, 2006.
17. **Olson JS, Foley EW, Rogge C, Tsai AL, Doyle MP, Lemon DD.** NO scavenging and the hypertensive effect of hemoglobin-based blood substitutes. *Free Radic Biol Med* 36: 685–697, 2004.
18. **Ortegon DP, Davis MR, Sampson JB, Dick E, Kashyap V, Kerby J.** Bovine hemoglobin-based oxygen-carrying solution (HBOC-201) improves flap survival in a rat model of epigastric flap failure. *Microsurgery* 26: 203–206, 2006.
19. **Privalle C, Talarico T, Keng T, DeAngelo J.** Pyridoxalated hemoglobin polyoxyethylene: a nitric oxide scavenger with antioxidant activity for the treatment of nitric oxide-induced shock. *Free Radic Biol Med* 28: 1507–1517, 2000.
20. **Raat NJH, Liu JF, Doyle MP, Burhop KE, Klein J, Ince C.** Effects of recombinant-hemoglobin solutions rHb2.0 and rHb11 on blood pressure, intestinal blood flow, and gut oxygenation in a rat model of hemorrhagic shock. *J Lab Clin Med* 145: 21–32, 2005.
21. **Sawyer GJ, Dong X, Whitehorne M, Grehan A, Seddon M, Shah AM, Zhang X, Fabre JW.** Cardiovascular function following acute volume overload for hydrodynamic gene delivery to the liver. *Gene Ther* 15: 452–462, 2007.
22. **Spahn DR, Kocian R.** Artificial O2 carriers: Status in 2005. *Curr Pharm Des* 11: 4099–4114, 2005.
23. **Tsai AG, Cabrales P, Manjula BN, Acharya SA, Winslow RM, Intaglietta M.** Dissociation of local nitric oxide concentration and vasoconstriction in the presence of cell-free hemoglobin oxygen carriers. *Blood* 108: 3603–3610, 2006.
24. **Vaslef SN, Kaminski BJ, Talarico TL.** Oxygen transport dynamics of acellular hemoglobin solutions in an isovolemic hemodilution model in swine. *J Trauma* 51: 1153–1160, 2001.
25. **Walsh MP, Marshall JM.** The early effects of chronic hypoxia on the cardiovascular system in the rat: role of nitric oxide. *J Physiol* 575: 263–275, 2006.
26. **Winslow RM.** Current status of blood substitute research; towards a new paradigm. *J Int Med Res* 253: 508–517, 2003.