Cardiovascular responses to oxygen inhalation after hemorrhage in anesthetized rats: hyperoxic vasoconstriction

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INHALATION OF 100% OXYGEN has been shown to improve survival in numerous animal models of hemorrhagic shock (6, 11, 20, 50), and this is the basis for the recommendation of oxygen administration in the clinical guidelines for the care of trauma victims (16). Although it has been clearly demonstrated that oxygen inhalation can improve tissue oxygenation (6, 50), it is not clear whether this is a direct result of increased oxygen content of blood or a secondary consequence of improved tissue perfusion. Absent the use of perfluorocarbons, significant hemodilution, or pulmonary compromise, hyperoxia causes little change in the oxygen content of blood. However, hyperoxia normally causes a vasoconstriction, which may itself provide a salutary effect by improving perfusion of some critical vascular beds through venoconstriction (9) and a favorable redistribution of the cardiac output (CO) (10).

Indeed, in most studies that have shown improved survival in controlled hemorrhage, the inhalation of normobaric oxygen caused an increase in systemic blood pressure (1, 9, 11, 15, 18, 37, 48, 50). Studies that have failed to show a survival benefit have generally not seen an increase in blood pressure (31, 55). The exception is in uncontrolled hemorrhage, where the increase in blood pressure can be detrimental by causing increased bleeding (47). Thus the results to date suggest that the oxygen-induced vasoconstriction and the increase in blood pressure are critical factors influencing the outcome of animals breathing oxygen after hemorrhage. However, most studies have only examined oxygen administration early after the start of hemorrhage, and the clinical therapeutic use of oxygen may occur much later. It is not known whether the timing of administration or the severity of shock influences the vasoconstriction and the increase in blood pressure. In the present study, we examined the blood pressure response to oxygen inhalation at various times after the onset of hemorrhage, primarily to determine the most beneficial conditions for its therapeutic use in trauma/hemorrhage. We detailed the cardiovascular responses involved in the early response to oxygen inhalation and explored some potential mechanisms. We also explored the stability of blood pressure response with respect to time and explored possible mechanisms for its change. The results demonstrate that an important action of oxygen is lost in late hemorrhage, indicating the need to reevaluate the risk versus benefit of oxygen use in late hemorrhage.

MATERIALS AND METHODS

Animal treatment. The experiments were conducted in conformity with an Institutional Animal Care and Use Committee-approved protocol. Animal handling and treatments were conducted in compliance with the Animal Welfare Act and other federal statutes and regulations related to animals and experiments involving animals, and they adhered to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council. The facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Male Sprague-Dawley rats were commercially obtained and quarantined for 10 days in a temperature- and light-controlled environment. Only animals demonstrating weight gain of 5–12 g over the previous day were used for experiments (animal weight between 350 and 450 g).

Surgical preparation. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium dissolved in 0.9% saline (50 mg/kg). Additional intraperitoneal injections of pentobarbital were administered as needed to maintain a surgical plane of anesthesia.

The trachea was cannulated with a PE-240 catheter to maintain free respiration. Unless specifically stated, ventilation was unassisted. A cannula (PE-50) was placed in the left femoral artery for monitoring the blood pressure and heart rate and for the periodic removal of blood samples. A second cannula (PE-50) was placed in a femoral artery for blood removal during the hemorrhage protocol. Core temperatures were monitored with a rectal thermost (model 49TA, Yellow Springs, Yellow Springs, OH) and maintained at 37°C by placing the animals under a heating lamp as needed. In experiments measuring CO, a cannula was placed in the right external jugular vein (PE-50).

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and advanced 30 mm to the right atrium for the injection of room temperature saline for the determination of CO, for drug administration, or for the measurement of central venous pressure. In those experiments where CO or contractility was measured, the right carotid artery was cannulated and used to position a thermistor (PE-10) in the ascending aorta for CO measurements or to position a cannula (PE-50) in left ventricle for the measurement of ventricular pressures. In these experiments, blood pressure was measured through a cannula placed in the other femoral rather than the carotid. Animals were treated with heparin (300 units at the start of the experiment and a subsequent dose of 200 units at 2 h).

**Hemodynamic and metabolic monitoring.** Blood samples (0.7 ml of blood replaced with normal saline) were obtained before hemorrhage in all animals. In most protocols, additional samples were obtained 3 min before oxygen inhalation and at the end of the oxygen breathing periods. The samples were analyzed for the $P_{aO_2}$, $P_{aCO_2}$, pH, blood sodium, potassium, chloride, calcium, osmolality, lactate, and hematocrit (NOVA Profile Analyzer, Nova, Biomedical, Waltham, MA). Blood pressure was monitored by a Statham pressure transducer. Animals with an initial mean arterial blood pressure (MABP) <90 mmHg were excluded from further study. A computerized data acquisition system recorded the MABP, systolic, and diastolic pressures every 5 s. Heart rates were determined by the Cardiomax II (model 85, Cardiomax II, Columbus Instruments, Columbus, OH) and recorded by hand immediately preceding and following oxygen administration.

CO was measured by thermal dilution (Cardiomax II). For each determination, 100 ml of room temperature saline were injected into the right atrium using an automatic injection syringe. CO was measured during the 3 min before oxygen challenge and during the last 1.5 min of oxygen inhalation. The results of repeat measurements (2 to 3) were averaged. Total peripheral resistance (TPR) was calculated by dividing the MABP by the CO.

Myocardial contractility was determined by detailed analysis of the pressure changes in the left ventricle. Pressures from the right atrium, the femoral artery, and the left ventricle were recorded at a sampling rate of 500 Hz. The respiratory phase was determined from changes in the central venous pressure. In the spontaneously breathing rat, inspiration was manifest in the central venous pressure waveform by a drop in pressure, and expiration was manifest by a return to the baseline pressure. Since the expiratory phase was three to four times longer than the inspiratory phase, the central venous pressure demonstrated a long plateau in the expiratory phase. Measurements of the first derivative of left ventricular pressure ($dP/dt$) were made at end expiration. Since maximal $dP/dt$ is sensitive to changes in afterload, myocardial contractility was determined from maximal $dP/dt_{max}$ divided by left ventricular pressure at that point in time (35). Measurements were averaged for each animal during the last 10 s of air breathing and the last 30 s of oxygen inhalation.

**Hemorrhage.** Blood was withdrawn or returned to the animal by use of a servo-controlled roller pump. MABP was lowered to a predetermined target pressure (30, 40, or 70 mmHg) by blood withdrawal gradually over a 15-min period; thereafter, the feedback control system maintained MABP at the target pressure by blood removal or return.

**Experimental design.** Thirteen different experiments were performed. Seven experiments examined the response to oxygen inhalation early after hemorrhage, detailing the cardiovascular responses and examining potential mechanisms. The remaining experiments examined the response late after hemorrhage and the potential influence of lactic acidosis on the response. The target pressures and number of animals involved in each experiment fall into five categories and are shown in Table 1.

**Gas challenges.** The animals normally breathed room air. At the time of a “gas challenge,” the inspired gas was changed to 100% oxygen humidified in a gas bubbler and supplied to the animal through a large-caliber (6 mm ID) nonobstructing tube that was positioned over the animal’s tracheotomy tube (2.44 mm OD). Feedback control of blood pressure was stopped 5 s before the gas challenge. Gas challenges lasted 4 min. The servo-control system was reactivated 3 min after the removal of oxygen. MABP was returned to the prechallenge pressure and maintained at this target until the next gas challenge. The change in MABP during the gas challenge was calculated by subtracting the average MABP during the last 30 s of air inhalation from the average MABP during the last 30 s of test gas inhalation.

The timing for the various gas challenges in animals hemorrhaged to a target pressure of 70 mmHg is shown in Fig. 1. The automatic feedback control of blood pressure was engaged when the blood pump

### Table 1. Experimental design

<table>
<thead>
<tr>
<th>Group</th>
<th>Target BP, mmHg</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls—breathing room air early after hemorrhage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td><strong>Effect of oxygen inhalation early after hemorrhage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A: MABP and CO responses (Table 2)</td>
<td>70</td>
<td>19</td>
</tr>
<tr>
<td>B: Myocardial contractility responses (Table 2)</td>
<td>70</td>
<td>9</td>
</tr>
<tr>
<td>C: Blood gas responses (Table 3)</td>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td>D: BP response-effects of changes in %inspired oxygen (Fig. 3)</td>
<td>70</td>
<td>7</td>
</tr>
<tr>
<td><strong>Exploration of potential mechanisms for early oxygen response</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E: BP response-effect of L-NAME (Fig. 4)</td>
<td>70</td>
<td>6</td>
</tr>
<tr>
<td>F: BP response-effect of ventilator control (Fig. 5)</td>
<td>70</td>
<td>11</td>
</tr>
<tr>
<td><strong>Early vs. late responses to oxygen inhalation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G: BP response-changes over time (Fig. 6)</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>H: BP response-early vs. at 10% return of PSBV (Fig. 7)</td>
<td>-40</td>
<td>10</td>
</tr>
<tr>
<td>I: BP response-early vs. at 139 min (Fig. 7)</td>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td>J: BP response-changes over 15 min</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td><strong>Exploration of potential causes for loss of oxygen response</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K: BP response-effect of lactate infusion (Fig. 8)</td>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td>L: BP response-effect of acid infusion (Fig. 8)</td>
<td>70</td>
<td>5</td>
</tr>
</tbody>
</table>

BP, blood pressure; MABP, mean arterial blood pressure; CO, cardiac output; L-NAME, N\textsuperscript{\textdegree}nitro-l-arginine methyl ester; PSBV, peak shed blood volume.
was on. During the first 15 min that the blood pump is engaged, the program withdraws blood at a rate determined to reach the target pressure by a linear decline in blood pressure. Thereafter, the feedback control is programmed to maintain the target pressure whenever the blood pump is engaged.

For comparisons of responses before hemorrhage to those early after hemorrhage to 70 mmHg (groups A, B, and C), oxygen was administered at period 1 and again at period 2, as illustrated in Fig. 1.

The experimental design to compare the effects of various concentrations of oxygen in the inspired gas (group D) followed the initial procedures shown in Fig. 1. The animals were hemorrhaged to 70 mmHg and given their initial gas challenge at period 2. Thereafter, the animals were given multiple challenges of oxygen at various concentrations. In one group, animals were first challenged with 100% oxygen and then subsequently challenged with progressively lower concentrations. Another group started breathing room air and was subsequently challenged with progressively higher concentrations of oxygen. Challenges occurred approximately every 7 min [4-min exposure to oxygen and 3 min to return to target pressure (pump on)].

Inspired oxygen concentration was determined by flowmeters.

For studies of the effects of various interventions (infusions or placing the animal on a ventilator, groups E, F, K, and L), oxygen was administered at periods 2 and 4, and the intervention occurred during period 3 as illustrated in Fig. 1.

Ventilator support. In one group of animals (group F), any change in ventilation was prevented by ventilator-assisted breathing. The animals were hemorrhaged to a target pressure of 70 mmHg, and they received an oxygen challenge at 5 min after reaching the target pressure. Thereafter, they were placed on a positive pressure ventilator (Rodent Ventilator model 683, Harvard Apparatus, Holliston, MA), and the volume and timing of ventilation were adjusted to approximate the ventilatory rate and chest wall expansion of the animal before being placed on the ventilator. A second oxygen challenge was then administered (period 4 in Fig. 1).

Test infusions. The effects of N\textsuperscript{6}-nitro-l-arginine methyl ester (l-NAME), lactate, and hydrochloric acid infusions on the oxygen response were evaluated in animals hemorrhaged to 70 mmHg (groups E, K, and L). Baseline response was tested early after hemorrhage (period 2 in Fig. 1). At 47 min, infusions were begun using the right atrial catheter. In the lactate infusion experiments, 0.2 ml of sodium lactate (600 mg/ml) was infused over 3 min (starting at 47 min), followed by a continuous infusion of 0.007 ml/min. In the acid infusion experiments, acid (2 N HCl in 0.5 N saline) was infused (1.3 ml over 10 min, period 3 in Fig. 1). At 60 min, the animals were again challenged with oxygen (period 4 in Fig. 1). Blood chemistries were drawn at the end of the gas exposures. In the l-NAME infusion experiments (group E), 20 mg/kg of l-NAME (10 mg/ml saline, pH 7.4) was infused over 5 min starting at 55 min, followed by a continuous infusion of 0.05 ml/min. At 65 min, the animals were again challenged with oxygen. (period 3 shown in Fig. 1 was extended in this group of animals). TPR was measured before and during each oxygen challenge.

Prolonged hypotensive periods (early vs. late responses). The timing for the various gas challenges in animals hemorrhaged to a target pressure of 40 mmHg is shown in Fig. 2. The initial events are the same as shown in Fig. 1, but the protocol differs after the initial oxygen challenge (period 2). Subsequent oxygen challenges were based on the status of the shed blood volume. Previous work by Connell et al. (17) has shown that animals at comparable shed blood volume status have very similar metabolic parameters. As shown in Fig. 2, during the initial time after period 2, blood had to be continually removed to maintain the target pressure of 40 mmHg. This results in the gradual increase in shed blood volume. After some period of time there is a change, and, thereafter, blood must be continually returned to the animal. During this later phase, shed blood volume continually decreases as blood is returned to the animal. The peak shed blood volume (PSBV) normally approximates 30 ml/kg, and it occurs at the time when TPR reaches its maximum (52) (Pearce FJ, unpublished observations).

Group G was hemorrhaged to a target pressure of 40 mmHg and received an oxygen challenge early after hemorrhage (period 2) and additional challenges when shed blood volume reached 50% and 75% of estimated PSBV as well as at the PSBV and at 10% return of PSBV.

Group H was hemorrhaged to a target pressure of 40 mmHg and had only two challenges of oxygen; early after hemorrhage (period 2) and at 10% return of PSBV (period 5 in Fig. 2). On average, these animals reached 10% return of PSBV at 139 min. For comparison with group H, group I was hemorrhaged to a target pressure of 70 mmHg and had only two oxygen challenges: early after hemorrhage and at 139 min (i.e., the experimental design was the same as Fig. 1, only with a longer period 3).

In group J, where animals were hemorrhaged to a target pressure of 30 mmHg, the responses changed so quickly that there was not sufficient time for the animals to be used as their own controls. Consequently, two groups of animals were examined. The first experiment followed the design of Fig. 1, and animals were given the oxygen challenge at period 2 (5 min after reaching target pressure). In the second group, animals were held at 30 mmHg for 15 min before oxygen inhalation.

Statistical analysis. Data are expressed as means ± SE. Experiments were analyzed by a Student’s t-test using paired analysis when comparing effects in the same animals after two different exposures to oxygen. In experiments using multiple, repeat measurements from the same animal, the planned comparisons were done with one-way analysis of variance. If the repeated-measures analysis of variance demonstrated significance, further analysis was done on a sample by sample basis using Fisher protected least significant difference post hoc analysis to determine which experimental groups significantly
Cardiovascular responses to oxygen after hemorrhage. Table 2 summarizes the results of experiments in which the changes in CO and MABP were measured during oxygen inhalation before and after hemorrhage (group A). Oxygen inhalation after hemorrhage resulted in a larger increase to TPR that was not seen before hemorrhage. There was also an increase in CO that can be explained by the increase in heart rate during oxygen inhalation. This increase in CO was not seen in animals before hemorrhage. In a separate group of animals, myocardial contractility was measured (group B). The increase in MABP was smaller in these animals. There was a small but significant increase in myocardial contractility during oxygen inhalation. There was no change in central venous pressure; however, there was an increase in end-diastolic pressure.

Preliminary experiments showed that the MABP was the same whether the inspired gas was room air or pressurized humidified room air. The increase in MABP while breathing room air early after hemorrhage (pump off) was 13 ± 4 mmHg when animals were hemorrhaged to target pressures of 70 mmHg (n = 5) and 40 mmHg (n = 5).

Blood pressure response to inspiration of oxygen at various concentrations. Figure 3 shows the increase in MABP during several exposures to oxygen of different concentrations (group D). All exposures occurred after hemorrhage to 70 mmHg. Each line connects the results from a single animal. The increase in MABP is expressed as a percentage of the change seen when this animal breathed 100% oxygen. Zero indicates that the blood pressure remained at 70 mmHg. The single point below 0 indicates that MABP fell below 70 mmHg during this exposure. Open symbols show the results of animals exposed to sequentially higher concentrations of oxygen. The closed symbols display results from animals given 100% oxygen as the first exposure and then exposed to sequentially lower concentrations. It can be seen that in all animals, near-maximal blood pressure response is seen at FIO2 of 60%.

Effect of l-NAME on the oxygen response. Preliminary studies revealed that a single bolus injection of 7.5 and 10 mg/kg had no effect on the oxygen response. Figure 4 shows the results of the injection of 20 mg/kg given as a single injection and followed by a slow continuous infusion (group E). The left panel shows that the change in MABP was similar before and after the infusion of l-NAME. TPR in-

Table 2. Measurements of oxygen-induced changes in cardiovascular function before and after hemorrhage

<table>
<thead>
<tr>
<th></th>
<th>Breathing Oxygen Before Hemorrhage</th>
<th>Breathing Room Air Early After Hemorrhage</th>
<th>Breathing Oxygen Early After Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP, mmHg</td>
<td>123±4.3 (17)</td>
<td>142.6±3.1* (17)</td>
<td>73±1.1§ (19)</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td></td>
<td></td>
<td>132.8±2.7* (19)</td>
</tr>
<tr>
<td>TRP, PRU</td>
<td>1.0±0.05 (17)</td>
<td>1.17±0.06 (17)</td>
<td>0.89±0.05 (19)</td>
</tr>
<tr>
<td>TRP, PRU</td>
<td></td>
<td></td>
<td>1.25±0.1* (19)</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>128.7±9.3 (17)</td>
<td>127.4±6.4 (17)</td>
<td>85.9±4.5§ (19)</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td></td>
<td></td>
<td>112.1±6.7§ (19)</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>418±12 (11)</td>
<td>412±12 (11)</td>
<td>306±10§ (13)</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
<td>367±13§ (14)</td>
</tr>
<tr>
<td>MYOCARDIAL CONTRACTILITY IN ANIMALS GIVEN OXYGEN CHALLENGE Early after hemorrhage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>72.8±1.3 (9)</td>
<td>118.1±2.9* (9)</td>
<td></td>
</tr>
<tr>
<td>Contractility, mm/mmHg</td>
<td>76.1±6.3 (9)</td>
<td>80.6±6.8§ (9)</td>
<td></td>
</tr>
<tr>
<td>Central venous pressure, mmHg</td>
<td>-0.7±0.7 (9)</td>
<td>-1.0±0.6 (9)</td>
<td></td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>4.9±1.5</td>
<td>8.2±1.6</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals (shown in parentheses). Measurements were taken just before oxygen administration and at the end of a 4-min exposure. MABP, mean arterial blood pressure; TRP, total peripheral resistance; LVEDP, left ventricular end-diastolic pressure; PRU, peripheral resistance units. Significantly different breathing oxygen vs. breathing room air: *P < 0.001; †P < 0.01; ‡P < 0.02. Significantly different from prehemorrhage baseline: §P < 0.001.
creased from 1.00 ± 0.15 to 1.40 ± 0.22 peripheral resistance units (PRU) \((n = 6)\) as a result of the infusion of L-NAME. However, as shown in the right panel, there was no difference in the increase in TPR during oxygen inhalation before versus after L-NAME infusion. PaO2 while breathing oxygen was similar before and after L-NAME infusion \([404 ± 35 \text{ vs. } 451 ± 9 \text{ mmHg (}n = 3)\].

### Blood gas changes during oxygen inhalation during baseline and early after hemorrhage

Table 3 shows the results from five animals \((\text{group C})\) given oxygen to breathe before hemorrhage \((\text{period 1 in Fig. } 1)\) and early after hemorrhage \((\text{period 2 in Fig. } 1)\). As expected, oxygen inhalation causes a large increase in PaO2, in both periods. Early after hemorrhage, there is a decrease in PaCO2, which is accompanied by an increase in pH. Breathing oxygen early after hemorrhage results in an increase in PaCO2.

### Effect of ventilator support on the oxygen response to oxygen inhalation after hemorrhage

Figure 5 shows the response to oxygen inhalation after hemorrhage to 70 mmHg in spontaneously breathing animals and animals on a ventilator \((\text{group F})\). PaO2 while breathing oxygen was similar before and after ventilator support \([434 ± 18 \text{ vs. } 439 ± 18 \text{ mmHg (}n = 8 \text{ and } 9, \text{ respectively})]\). However, the change in PaCO2 was different in the two groups. After hemorrhage, the PaCO2 in spontaneously breathing animals was 26.4 ± 2.7 \((n = 9)\) and the PaCO2 increased to 36 ± 2 \((n = 9)\) during oxygen inhalation. In animals on a ventilator, the initial PaCO2 was 26 ± 1 \((n = 8)\), and this remained at 26 ± 1 \((n = 8)\) during oxygen inhalation. In the left panel of Fig. 5 is shown that the increase in MABP was similar in spontaneously breathing animals to those on ventilator support. In the right panel of Fig. 5 is shown that the increase in TPR was also similar in both groups. With animals that breathed room air, the TPR was 0.94 ± 0.11 PRU in the spontaneously breathing animals and 1.4 ± 0.2 PRU in the animals on a ventilator.

### Oxygen response in the later phases of shock

Figure 6 examines the response to inhalation of 100% oxygen at various
points during isobaric hemorrhage (group G). All animals in this group were hemorrhaged to 40 mmHg. It can be seen that the blood pressure response to oxygen inhalation early after hemorrhage is very similar in this group as the response seen in animals hemorrhaged to 70 mmHg (Table 2). Likewise, animals hemorrhaged to 30 mmHg MABP had a similar response to oxygen inhalation early after hemorrhage [55 ± 10 mmHg (n = 5)] (not shown).

In Fig. 6 there is a gradual loss of the oxygen response from 52.5 ± 7.1 mmHg early after hemorrhage to 11.5 ± 6.1 mmHg at 10% return of PSBV (n = 9). The responses at PSBV and 10% return of PSBV differ significantly from the early response (P < 0.004 and P < 0.001, respectively). The decrease in oxygen response occurred at the same time that there was a gradual increase in blood lactate from 3 ± 0.3 at the early time point to 11.8 ± 2.3 mmol/l at 10% return of PSBV (n = 7). The blood lactate levels at 75% of PSBV and thereafter are significantly different from the levels at the early time point.

Figure 7 shows the results of two experiments. The two left-hand bars essentially reproduce the previous experiment, except that the number of oxygen exposures was limited to two, one early and one at 10% return of PSBV (group H). Just as shown in Fig. 6, the oxygen response was lost when 10% of the PSBV was returned to the animal. The increase in MABP with oxygen averaged 65.9 ± 9.8 mmHg at the early time point and 0.5 ± 3.3 mmHg at 10% return of PSBV (P < 0.001, n = 10). There was no difference in PaO2, during oxygen inhalation at the two time points. At the early time point, oxygen inhalation caused the PaO2 to increase from 103 ± 5 to 379 ± 31 mmHg. At 10% return of PSBV, oxygen inhalation caused PaO2 to decrease significantly from 116 ± 6 to 421 ± 11 mmHg (n = 8). The hematocrit was 37.5 ± 0.0% at the early time point and 37.4 ± 1.0% at 10% return of PSBV. Table 4 shows the changes in blood lactate, pH, calcium, glucose, osmolality, and blood electrolytes during oxygen exposure at the two time points in this protocol (n = 8). Similarly to Fig. 6, the blood lactate increased significantly from 3.9 to 15.8 mmol/l. In the animals in late shock at 10% return of PSBV, the correlation of blood pressure while breathing oxygen to the lactate level is R = 0.814, P < 0.01. Concomitant with the increase in blood lactate, there was a significant decrease in blood pH from 7.42 to 7.22. There were no significant changes in the other measured parameters (except bicarbonate, which reflects the changes in blood pH when changing from 24.7 ± 0.8 to 7.7 ± 1.3 mmol/l).

On average, it took 139 min for animals to reach 10% return of PSBV when they were held at 40 mmHg. Fig. 7, right, shows the results when animals were held at 70 mmHg for 139 min (group I). They did not significantly increase their blood lactate (1.1 ± 0.1 to 2.1 ± 0.5 mmol/l) or lower their blood pH (7.42 ± 0.01 to 7.46 ± 0.02), and they only show a very modest decrease in their oxygen response from 68.9 ± 4.0 to 56.8 ± 3.5 mmHg (P < 0.02, n = 5). The arterial PaO2 on oxygen as quite similar at the two time points (413 ± 25 early and 442 ± 26 mmHg at 10% return of PSBV).
hemorrhage of 55/H11006 at 15 min) or PaO2 during oxygen inhalation (381 difference in blood pH (7.33 P after hemorrhage (right axis)). The left panels display results from animals hemorrhaged to 40 mmHg. Oxygen was administered early after hemorrhage and when 10% of PSBV had been returned to the animal (average of 139 min). The right panels display results from animals hemorrhaged to 70 mmHg. Oxygen exposures occurred early after hemorrhage and at 139 min after hemorrhage. Between oxygen exposures, the MABP was maintained at the target pressure by blood withdrawal or return by using computer-regulated feedback control.

If animals were hemorrhaged to 30 mmHg (group J), there was a significant loss of oxygen response after only 15 min. Animals that received an oxygen challenge 15 min after hemorrhage to 30 mmHg increased MABP only 19.7 ± 4.7 mmHg (P < 0.001, n = 5) as compared with the response early after hemorrhage of 55 ± 10 mmHg. The two groups differed in blood lactate [3.9 ± 0.2 early (5 min) and 6.5 ± 0.3 at 15 min after hemorrhage (P < 0.0001)]. However, there was no difference in blood pH (7.33 ± 0.01 at 5 min and 7.38 ± 0.01 at 15 min) or PaO2 during oxygen inhalation (381 ± 47 at 5 min and 434 ± 24 mmHg at 15 min).

Infusions of lactate or acid. Figure 8 shows the results of systemic infusions on the oxygen response. In both cases, the animals were hemorrhaged to 70 mmHg. In each panel, the bars on the left show the results before infusion and the bars on the right show the results in the same animals after the systemic infusion.

In Fig. 8, the two bars on the left show the results before and after the infusion of sodium lactate at a rate that increased blood lactate to levels approximating those seen at 10% return of PSBV (group K). Shown on the line graph using the right-hand axis, lactate increased to 13.5 ± 1.5 mmol/l after the lactate infusion. The increase in blood lactate alone did not alter the rise in MABP during oxygen inhalation. MABP increased 53.1 ± 1.8 mmHg during oxygen inhalation before the lactate infusion and 48.2 ± 3.8 after the infusion (n = 5, not significant). PaO2 while breathing oxygen was similar before and after lactate infusion [432 ± 26 vs. 482 ± 33 mmHg]. The infusion of sodium lactate did not lower blood pH. pH before lactate was 7.41 ± 0.03, and it changed to 7.53 ± 0.02 after the lactate infusion. In Fig. 8, the two bars on the right show the results before and after the infusion of hydrochloric acid (group L) at a rate that lowered the blood pH to levels approximating or exceeding that seen at 10% return of PSBV in Fig. 5. The infusion of acid lowered pH from 7.43 ± 0.01 to 7.13 ± 0.04. This was associated with some decrease in the oxygen response from 61.4 ± 5.2 to 42.9 ± 1.3 mmHg (n = 5, P < 0.02). PaO2 while breathing oxygen was similar before and after acid infusion (412 ± 21 vs. 453 ± 20 mmHg). Lactate did not increase with the acid infusion (0.9 ± 0.1 before and 0.9 ± 0.1 after).

DISCUSSION

Response to oxygen in early hemorrhage. The most striking finding of the current study is that there is a very large increase in blood pressure (60 ± 3 mmHg) in response to oxygen inhalation early after hemorrhage to a target pressure of 70 mmHg (Table 2). This large increase in MABP occurs despite the absence of arterial hypoxemia, and it has been seen in several other studies of oxygen inhalation in early hemorrhage (1, 10, 18). A similar response to oxygen inhalation early after hemorrhage is also seen in animals hemorrhaged to target pressures of 40 mmHg (increase of 60 ± 4.3 mmHg, Fig. 6) and 30 mmHg (increase of 54.9 ± 10 mmHg). In a separate study (49), a similar response was also seen in conscious rats hemorrhaged to 40 mmHg [increase of 50 ± 5 mmHg (n = 13)]. In the present study, the change in MABP is mediated primarily through a substantial increase in TPR, although there is some increase in CO resulting from the increase in heart rate and a small increase in myocardial contractility (Table 2).

Early during hemorrhage, there is a decrease in heart rate from 418 to 306 beats/min (Table 2). This is consistent with hemorrhage-induced sympathoinhibition (5, 43). Several central pathways have been implicated in hemorrhage-induced sympathoinhibition, including nitric oxide (NO) or related compounds (33). In support of this mechanism is the recent finding by Atkins et al. (4) that there is a large increase in NO

Table 4. Metabolic parameters in animals held at 40 mmHg until requiring 10% return of PSBV

<table>
<thead>
<tr>
<th></th>
<th>Early After Hemorrhage</th>
<th>At Return of 10% of Shed Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate, mmol/l</td>
<td>3.8 ± 0.06</td>
<td>15.8 ± 1.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.42 ± 0.03</td>
<td>7.22 ± 0.04</td>
</tr>
<tr>
<td>Sodium, mmol/l</td>
<td>140 ± 1</td>
<td>142 ± 0.8</td>
</tr>
<tr>
<td>Potassium, mmol/l</td>
<td>4.7 ± 0.2</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>Chloride, mmol/l</td>
<td>110 ± 0.6</td>
<td>114 ± 0.9</td>
</tr>
<tr>
<td>Calcium, mmol/l</td>
<td>1.28 ± 0.01</td>
<td>1.26 ± 0.02</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>239 ± 2.2</td>
<td>204 ± 58</td>
</tr>
<tr>
<td>Osmolality, mosmol/kgH2O</td>
<td>283 ± 1</td>
<td>285 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE for the averages of measurements from blood samples taken early after hemorrhage and at the point when 10% of the peak shed blood volume (PSBV) had been returned to the animals. All animals were hemorrhaged to 40 mmHg. *Significant with Bonferroni correction (P < 0.0063).
production in early hemorrhage. The subsequent increase in heart rate during oxygen inhalation after hemorrhage suggests that there was a partial reversal of the sympathoinhibition by oxygen. This conclusion is supported by the increase in myocardial contractility and by a separate study (3) that showed a fall in plasma norepinephrine levels during hemorrhage, followed by a subsequent increase during oxygen inhalation.

A decrease in NO production could potentially explain the increase in heart rate and increase in TPR through reversal of sympathoinhibition. Decreased NO production would also increase TPR through the loss of the direct vasodilator action of NO. To explore the potential role of NO in the oxygen-induced increase in blood pressure, we examined the effect of the nonselective NO synthase inhibitor L-NAME. We used a dosage of L-NAME that we had previously shown decreases NO production in this phase of hemorrhage (4). L-NAME had a systemic effect as evidenced by the increase in TPR and the need to withdraw additional blood to return to the target pressure. However, L-NAME did not block the response to oxygen inhalation, indicating that this response does not directly involve NO synthase. However, this result does exclude the involvement of NO produced from nitrite (26, 32, 38) or S-nitrosothiols produced from nitrite (2). These pathways are sensitive to tissue oxygenation (i.e., NO or S-nitrosothiol production decreases with better tissue oxygenation), but they would not be influenced by a short duration NO synthase inhibition. The failure of NO synthase inhibition to change this response could also indicate that there is a redundancy in endogenous cardiovascular regulation that cannot be blocked by a single inhibitor or that other mechanisms are involved.

We have explored one other potential mechanism of vasocostriction that is a response to the increase in PCO\textsubscript{2}. Table 3 shows that oxygen inhalation after hemorrhage is associated with an increase in PCO\textsubscript{2}. We explored the potential role of this ventilatory change by the experiment shown in Fig. 5, in which the animal’s ventilation was controlled by a mechanical ventilator during the second exposure to oxygen. The manipulation prevented the increase in PCO\textsubscript{2}, but it had no effect on the blood pressure response to oxygen or the changes in TPR. We conclude that the change in PCO\textsubscript{2} did not cause the oxygen-induced vasocostriction.

The cause of the changes in ventilation is unknown. Hypoxic regulation of ventilation has recently been shown to involve S-nitrosothiols (22), and increased production of S-nitrosothiols in hemorrhage could account for the increased ventilation in early hemorrhage (22). Likewise, the inhibition of ventilation during oxygen inhalation could be caused by a decrease in the production of S-nitrosothiols, but increases in MABP (7) and norepinephrine infusions (13) have also been shown to inhibit ventilation.

Response to oxygen in prolonged hemorrhage. An unexpected result of this study is that the pressor response to oxygen gradually decreases if the MABP remains at 40 mmHg for a long period of time. (Figs. 6 and 7). The results of a recent study by Gunther et al. (24) may indicate that the changes in heart rate and CO are lost before the changes in TPR (24). The cause for the loss of oxygen response is not known.

In the present study, Fig. 7 indicates that these changes are not just time related, since the oxygen response was preserved over the same time period if the animal was maintained at 70 mmHg. Moreover, the loss of response could occur very rapidly if the animal was maintained at 30 mmHg. The loss of response does correlate with increasing levels of lactic acid (Figs. 6 and 7 and Table 4). However, increasing blood levels of lactate are not sufficient to cause the loss of the oxygen response (Fig. 8), although it can be partially reproduced by acid infusion. The loss of hyperoxic vasoconstriction could indicate that oxygen inhalation fails to improve tissue oxygenation in late shock. It is also possible that shock and/or acidosis disrupts the mechanisms of hyperoxic vasoconstriction. Numerous mechanisms have been proposed for hyperoxic vasoconstriction (12, 21, 23, 34, 41, 45, 46, 53), and several could be influenced by the alterations that occur in prolonged hemorrhage, such as changes in the redox status within the cell (54), or opening of potassium channels in vascular smooth muscle (56). The oxygen response could also be influenced by the decrease in the oxidase activity of ceruloplasmin, which occurs in prolonged hemorrhage (as evidenced by a decrease in its electron paramagnetic resonance signal (4)). The oxidase activity of ceruloplasmin can help to generate nitrite (44), and this pathway may be important for oxygen-sensitive production of NO (32, 38) or S-nitrosothiols from nitrite (2).

Mechanisms of beneficial effect and potential dangers of oxygen inhalation. The results of the current study suggest that the improved survival seen with oxygen inhalation early after hemorrhage (1, 9, 11, 18, 37, 48, 50) results from two effects: 1) reversal of sympathoinhibition and 2) oxygen-induced vasoconstriction. It should be noted that the reversal of sympa-
thoinhibition and improved tissue oxygenation can also be accomplished by fluid resuscitation in early hemorrhage; therefore, the greatest therapeutic benefit of oxygen may be its rapid onset of action. Oxygen inhalation is also beneficial in the case of extreme hemodilution induced by fluid resuscitation from significant hemorrhage (25, 39, 40).

The current study also demonstrated that, after prolonged hypotension without fluid resuscitation, oxygen inhalation failed to increase blood pressure. The results raise the concern that oxygen inhalation may be less beneficial in late shock. Although oxygen inhalation is normally thought to be innocuous, hyperoxia has been shown to increase reperfusion injury in isolated hearts and patients undergoing coronary artery bypass (27, 30). Much like the ischemia-reperfusion injury of coronary bypass, hemorrhage-resuscitation also causes oxidative injury as evidenced by free radicals detectable by electron paramagnetic resonance (Gorbunov NV and Atkins JL, unpublished observations) and by the beneficial effects of radical scavengers (8, 14, 19, 28, 29, 36, 42, 51). Takasu et al. (50) have recently raised the concern that oxygen inhalation in hemorrhage may increase free radical damage on the basis of their findings that 50% FIO2 was beneficial, whereas 100% FIO2 was not. Their findings suggest that we should carefully evaluate the dosage of administered oxygen, and the current results suggest that we should carefully evaluate the timing of oxygen administration and specifically evaluate the risk versus benefit of oxygen administration in late hemorrhage.

Summary. Oxygen inhalation causes a large increase in blood pressure early after hemorrhage but not late after hemorrhage. Since the therapeutic benefit of oxygen inhalation seems to correlate with its pressor effect in hemorrhage, the results suggest that the risk versus benefit of oxygen inhalation should be examined in late hemorrhage.

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The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense.

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