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Early Physiological Responses to Hemorrhagic Hypotension

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Abstract

The identification of early indicators of hemorrhagic hypotension (HH) severity may support early therapeutic approaches and bring insights into possible mechanistic implications. However, there are few systematic investigations of physiological variables during early stages of hemorrhage. We hypothesized that, in certain subjects, early physiological responses to blood loss are associated with the ability to survive hemorrhage levels that are lethal to subjects that do not present the same responses. Therefore, we examine the relevance of specific systemic changes during and after the bleeding phase of HH. Stepwise hemorrhage, representing pre-hospital situations, was performed in 44 rats and measurements were made after each step. Heart and respiratory rates, arterial and venous blood pressures, gases, acid-base status, glucose, lactate, electrolytes, hemoglobin, O₂ saturation, tidal volume and minute volume were measured before, during and after bleeding 40% of the total blood volume. Fifty percent of rats survived ≥ 100 min (survivors, S); others were considered nonsurvivors (NS). Our findings were: 1) S and NS subjected to a similar hemorrhage challenge showed significantly different responses during non-lethal levels of bleeding; 2) survivors showed higher blood pressure and ventilation than NS; 3) while pH was lower in NS at later stages, changes in bicarbonate and base excess occurred already during the hemorrhage phase and were higher in NS; 4) plasma K⁺ levels and glucose extraction were higher in NS. We conclude that cardio-respiratory and metabolic responses, essential for the survival at HH, can differentiate between S and NS even before a lethal bleeding was reached.

Keywords

glucose; minute ventilation; oxygenation; rats

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The material presented in this report is original and has not been submitted for publication elsewhere other than in abstract form.

INTRODUCTION

Delayed interventions in hemorrhaged subjects may occur in both civilian and military scenarios¹ and early indicators of severity in hemorrhagic hypotension (HH) could be useful to identify the mechanisms behind the ability to survive HH. Nevertheless, in most HH studies, the first post-hemorrhage measurement is typically performed 15–30 min after the end of bleeding^{2–4}. However, it is conceivable that some of the physiological responses that would eventually lead to survival could be present even before a lethal hemorrhage volume is reached. Therefore, data collected after HH is fully established may result in missing critical early physiological responses.

Our previous studies demonstrated that certain variables can distinguish between survivors and nonsurvivors of prolonged hemorrhagic shock^{5, 6}. In a constant-pressure model of HH, we found that variables related to respiration, oxygenation and tissue ischemia were different between S and NS⁶. Several studies have shown that hypoperfusion-related variables, such as arterial pressure, pH, bicarbonate, base excess and lactate, may be indicators of the severity of hemodynamic decompensation^{7, 8} but also have shortcomings⁹.

Clinical studies found that certain blood biochemical variables such as glucose and K⁺ were also associated with poor outcome in HH. Insulin therapy aiming at the maintenance of a normal blood glucose concentration has been shown to significantly reduce morbidity and mortality among critically ill patients¹⁰. The hypotension of the decompensatory phase of HH reflects decreases in vascular resistance⁵ that may be mediated in part also by potassium channels. Experimental studies using glibenclamide, a drug which blocks the K_{ATP} channel, showed an increase in MAP during HH and endotoxemia^{11, 12}, but failed to counteract arterial hypotension in human septic shock¹³.

Based on previous studies, specific hemodynamic, respiratory and biochemical (metabolic) variables are good candidates to differentiate between S and NS already during hemorrhage induction, revealing aspects of the mechanisms responsible for surviving a blood loss. The current study was designed to characterize these parameters, grouping the findings in S and NS categories. Our hypothesis was that increases in blood oxygenation and changes in associated systemic variables due to HH play a major role in the ability to discriminate S from NS *before* hemorrhage can be considered lethal. We also hypothesised that glucose extraction provides better discriminating power to differentiate NS and S than its arterial and venous values. Therefore, we performed a stepwise target-volume hemorrhage where 40% of the estimated total blood volume was withdrawn using a protocol of three successive hemorrhage steps. Arterial and venous blood samples were collected before, during and after the hemorrhage process, while respiratory (rate, tidal and minute volumes) and cardiovascular (blood pressures, heart rate) variables were continuously recorded throughout the experiment.

MATERIALS AND METHODS

Experimental animals

This study was approved in advance by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and conforms to the Public Health Service Policy on Humane Care and Use of Laboratory Animals (August, 2002) and the American Physiological Society's Guiding Principles in the Care and Use of Animals. We used forty-four male Sprague-Dawley rats (Harlan, Indianapolis, IN) averaging between 8–10 weeks old and weighing 301 ± 5 g (mean ± SEM). Rats were housed in plastic cages placed in a facility with a 12 h light/dark cycle, constant temperature (21 – 23°C) and humidity (48 – 50%) and maintained for an adaptation period of one week. They were fed a commercial diet and had free access to water.

Hemodynamic and respiratory measurements

The animals were subjected to tracheotomy and catheterizations under 2 % Isoflurane anaesthesia, followed by constant intravenous infusion ($0.24 - 0.36 \text{ mg kg}^{-1} \text{ h}^{-1}$) of alfaxalone/alfadolone acetate (Saffan, Schering-Plough Animal Health, Welwyn Garden City, England). The left femoral vein was cannulated with PE-50 tubing for this purpose. The right carotid artery was cannulated with PE-50 tubing filled with a heparin-PBS solution (10 i.u. ml^{-1} heparin), and connected to a pressure transducer to continuously measure arterial blood pressure (AP). The right jugular vein was cannulated with PE-90 tubing advanced to the entrance of the right atrium. This line was used to collect central venous blood samples and for recording central venous blood pressure (CVP). The left femoral artery was cannulated and connected to two microprocessor-controlled infusion/withdrawal syringe pumps (model PHD2000, Harvard Apparatus, Holliston, MA). The same artery was used to hemorrhage the animal (see below) and to collect blood samples. All catheters were flushed after blood collection with a volume of heparinized PBS (10 i.u. ml^{-1} heparin) no greater than the measured dead volume of the catheters to inhibit formation of clots. In order to monitor minute ventilation, a Y-shaped tube was inserted into the trachea and the expiratory limb of the tube was connected to a one-way valve and to a heated pneumotach attached to a differential pressure amplifier (Biopac Systems, Inc., Goleta, CA). The core temperature was monitored and maintained at $36.5 - 37.0^\circ\text{C}$ using a thermostatically-controlled heating blanket (Harvard Apparatus, Holliston, MA). Phosphate buffered saline (PBS) was administered at room temperature ($23 \pm 2^\circ\text{C}$) as hydration fluid before ($5 \text{ ml kg}^{-1} \text{ i.p.}$) and after ($5 \text{ ml kg}^{-1} \text{ i.p.}$) muscle exteriorization. Animals were not heparinized and breathed room air spontaneously.

Blood-gas, hematological, and biochemical measurements

Blood analyses were performed in all animals from paired arterial and venous samples (0.1 ml each) collected at various time points using heparinised glass capillary tubes (Clinitubes, D957G-70-100, Radiometer, Copenhagen, Denmark). All blood samples were immediately replaced by an equal volume of PBS (baseline) or blood (post-hemorrhage). Blood glucose, potassium, sodium, calcium, lactate, bicarbonate, base excess (BE), PO_2 , PCO_2 and pH were measured with a blood gas analyzer (ABL 725, Radiometer, Copenhagen, Denmark). The glucose extraction was calculated as glucose AV difference divided by arterial glucose. Total hemoglobin (Hb) concentration and Hb O_2 saturation were measured with a multiwavelength CO-oxymer adjusted for the oxyhemoglobin dissociation curve of rat (OSM3, Radiometer, Copenhagen, Denmark). Systemic hematocrit (HCT) was measured from arterial blood samples using a micro centrifuge (MicroMB, Thermo Electron Co., Beverly, MA).

Inclusion criteria

The following criteria had to be met by each animal to be subjected to hemorrhage: 1) baseline mean arterial pressure (MAP) above 90 mm Hg , 2) starting bicarbonate between 20 and 30 mmol l^{-1} , 3) arterial O_2 saturation above 90% and 4) Hb concentration above 10 g dl^{-1} . This helped to minimize the effects of initial dehydration, excessive blood loss during surgical preparation, hemodilution due to excessive catheter flushing and accidental food/water deprivation prior to surgery. Detailed examination of the experimental records showed that, in 3 cases, MAP dropped below 30 mmHg due to poor handling during blood sampling and temperature probe placement. These experiments were not included in the analysis. Data from all other animals that were alive by the end of the final hemorrhage step (see below) are being reported in this study.

Hemorrhage

The final target shed blood volume was calculated as 40% of total blood volume, which in turn was estimated as 6% of body mass. The hemorrhage was performed in three steps using the

following target volumes and blood withdrawal rates: step 1, 25% at 0.5 ml min^{-1} ; step 2, 10% at 0.2 ml min^{-1} ; and step 3, 5% at 0.1 ml min^{-1} . Animals were hemorrhaged using a double-lumen catheter (Braintree Scientific, Inc., Braintree, MA) for simultaneous bleeding and infusion of sterile sodium citrate solution (Baxter Healthcare Corp., Deerfield, IL) using two synchronized syringe pumps. Since the tip of the citrate catheter was about 5 mm proximal from the withdrawal site, infusion of citrate during blood withdrawal provided maintenance of catheter patency and preservation of shed blood. Hemorrhage was paused immediately if MAP fell to less than 40 mmHg and was resumed when MAP stayed above 45 mmHg for 15 s or more. Moreover, bled volume was adjusted to compensate for the blood volumes collected for sampling (blood analysis).

Protocol

After at least a 20-min stabilization period, baseline measurements were begun by obtaining blood samples for determination of arterial and venous blood gases and chemistry. During each blood collection, a time mark was placed in the computer recording of the systemic parameters in order to precisely match the data being collected. A second set of baseline measurements was performed 20 min later. After the second sampling for baseline, hemorrhage was performed. The time that the hemorrhage process started is considered as time point $t = 0$. Systemic blood gases and metabolic parameters, along with systemic hemodynamic, were measured after the first hemorrhage step (10 min), after the second step (20 min), and immediately after target hemorrhage volume was reached (28–29 min). Additional measurements were made 5 and 30 minutes later. Animals surviving over 100 minutes were considered survivors and were then included in a separate series of experiments, not reported here. No additional fluid infusion was used to maintain MAP. We have previously reported that control animals subjected to all procedures described above except hemorrhage showed relatively stable physiological parameters throughout an experimental period of five hours 6. Therefore, additional control experiments did not seem required in the current study because 1- the preparation of this study is very similar to the one described earlier 6; 2- the experimental period was shorter (approximately 2 hours); and 3- all baseline values were within the previously reported ranges.

Data acquisition and analysis

The amplified outputs from the pneumotach and from the pressure transducers (DA100C, Biopac Systems, Goleta, CA) were connected to a desktop computer for continuous on-line data acquisition at a rate of 200 Hz (Acqknowledge 3.9.0 + MP150 hardware and software; Biopac Systems, Goleta, CA). The inspiratory phase of each respiratory cycle generated negative pressure changes in the CVP tracings, easily distinguishable from right atrial pressure changes. Respiratory rate (RR) was calculated as the reciprocal of the interval between these successive negative pressure peaks in the CVP tracings, as well as between the flow peaks from the pneumotach tracings. Tidal volume (TV) was calculated on-line from the software auto-detection of the positive peak of the lung volume curve (estimated from the pneumotach signals). Systolic, diastolic, pulse and mean arterial pressures were calculated from the original digitized traces of AP. Heart rate (HR) was obtained on-line from software auto-detection of the rising arterial pulse wave after baseline removal and recorded continuously. All off-line calculations were based on one-min segments of the digitized signals, taken as close as possible to the blood collection time points. The mean minute ventilation was calculated as TV multiplied by RR. Arterial (CaO_2) and venous (CvO_2) O_2 contents were calculated as:

$$\begin{aligned}\text{CaO}_2(\text{ml dl}^{-1}) &= (\text{Hb} \bullet 1.34 \bullet \text{SaO}_2) + (0.003 \bullet \text{PaO}_2); \\ \text{CvO}_2(\text{ml dl}^{-1}) &= (\text{Hb} \bullet 1.34 \bullet \text{SvO}_2) + (0.003 \bullet \text{PvO}_2)\end{aligned}$$

in which SaO_2 and SvO_2 are the arterial and venous O_2 saturations, respectively, and PaO_2 and PvO_2 are the arterial and venous O_2 tensions, respectively. The O_2 extraction ratio (O_2 ER) was calculated, in percentage, as the difference of CaO_2 and CvO_2 divided by CaO_2 . Survival time was measured in minutes for each animal from time when hemorrhage was initiated to either failure (death) or censoring (animal survived to 100 min from the onset of hemorrhage).

Statistics

Data analysis was performed with a statistical software package (SigmaStat for Windows, Jandel Scientific). Data are presented as mean $\pm$ one standard error of the mean. The deviation from a Gaussian distribution was tested for all systemic data from all groups using the Kolmogorov-Smirnov test. When the distribution could not be fitted by a Gaussian distribution, Mann-Whitney and Kruskal-Wallis tests were used for comparisons among 2 and more than 2 groups, respectively. When parametric tests were found adequate, repeated-measures analysis of variance was followed, where appropriate, by the Holm-Sidak t-test and by the Student-Newman-Keuls method for multiple comparisons. A level of $p < 0.05$ was considered significant.

RESULTS

All baseline values were within the previously reported ranges for animals subjected to a similar surgical preparation. As described in the preceding section, control animals subjected to all procedures except hemorrhage showed relatively stable physiological parameters throughout an experimental period of five hours⁶. Since the preparation of this study is very similar to the one described earlier and the experimental period of the data being reported was shorter, additional control experiments were not performed. Animals subjected to hemorrhage showed a relatively wide range of responses. Some of them decompensate quite early and died as early as 41 minutes following the start of blood withdrawal. These animals were also included in the present analysis.

In order to examine the data, a cutting point was established based on a mortality rate of 50%, as recommended previously¹⁴. An equal number of survivors (S) and nonsurvivors (NS) were obtained using 100 min after start of hemorrhage (or 70 min after end of hemorrhage) as a cut point. From a total of 44 animals subjected to hemorrhage, 22 rats survived more than 100 min (S) and 22 rats died in less than 100 min after hemorrhage was initiated. To avoid any bias, this was done before the analysis of the physiological parameters was performed. Technical issues resulted in some measurements being discarded and some animals dropped out (died) over the study protocol. Therefore, the number of measurements per variable at each time point varied among the 40 different variables that were evaluated in this study. For all variables, the mean at any given time point was calculated based on at least 8 animals.

The volumes and respective bleed times for each hemorrhage step are depicted in Figure 1. In each group, animals were bled within similar times by similar blood amounts reaching an average final volume of $24.2 \pm 0.4 \text{ ml kg}^{-1}$ (40% of the estimated total blood volume). The mean hemorrhage time to reach the target volume was $29 \pm 1 \text{ min}$ for all animals. The mean time from start of hemorrhage to death was $72 \pm 4 \text{ min}$ for NS (range: 41 – 96 min).

Respiratory and hemodynamic responses

Technical issues allowed collecting systemic data on 37 rats (17 survivors and 20 nonsurvivors) from the overall pool of 44. Hemodynamic and cardiac data from these hemorrhaged animals are shown in Table 1. Starting already at step 1 of hemorrhage, survivors showed higher levels of MAP, DP and SP than nonsurvivors. Pulse pressure and CVP (data not shown) progressively

decreased but were not different between NS and S throughout the experiment. Differences did reach statistical significance for heart rate only at $t = 59$ min (data not shown). Respiratory responses were also different between S and NS, sometimes as early as after the first step of hemorrhage. Figure 2 shows that the RR was significantly decreased in NS only after the hemorrhage ended. Although, tidal volume and minute ventilation were systematically higher for S animals, from the start of the hemorrhage period throughout the observation time, statistical significance between S and NS was only showed at 20 min and 34 min for TV and 12 min and 34 min for MV.

Blood sample measurements were performed in all animals subjected to hemorrhage. The biochemical values of arterial and venous blood showed that, despite the better respiratory response of S animals, oxygenation variables such as PO_2 , SO_2 and O_2 content were significantly different from NS only at certain time points (Table 2). Venous O_2 content was significantly lower and O_2 extraction ratio was significantly higher for NS after the second bleeding step (Figure 3). Changes in Hb concentration and hematocrit (Figure 4) showed a hemodilution that started in the first step of hemorrhage for all animals, but was more intense for NS during and immediately after the bleeding. However, no differences in hemoglobin concentration and hematocrit were found between S and NS at later stages.

The development of acidosis with some compensation is characterized by the data shown in Table 3 and Figure 5. Hemorrhaged animals exhibited decreased pH, PCO_2 , BE and HCO_3^- with simultaneous increase in lactate. The development of acidosis in NS was delayed until later stages as the arterial pH was only significantly lower than S at $t = 59$ min. Moreover, the pH of S animals was never significantly different from baseline values (Table 3). The time course of some compensatory mechanisms for keeping the pH close to normal can be appreciated considering the graphs in Figure 5. Changes in bicarbonate, BE and lactate were initiated as soon as the hemorrhage started and were more pronounced in NS. Again, similar values were found for venous blood.

Blood glucose and electrolytes

Changes in plasma K^+ levels followed a very distinctive pattern as illustrated in Figure 5. At all time points during hemorrhage and thereafter, NS had a higher K^+ level than that of S animals, the difference reaching 3 mmol l^{-1} . Arterial and venous glucose levels (Figure 6) were also significantly different between S and NS later in HH, nonsurvivors showing arterial hypoglycaemia at later stages. The calculated arteriovenous difference in glucose was remarkably different between S and NS during hemorrhage. The average calculated glucose extraction during baseline was $3 \pm 2 \%$ and $6 \pm 2 \%$ for survivors and nonsurvivors, respectively. During hemorrhage, the extraction changed to 15 ± 4 and $-21 \pm 14 \%$ for S and NS, respectively ($p < 0.05$). After hemorrhage, the glucose extraction was similar for S and NS. Transient slight hyponatremia (5% decrease from baseline, $0.05 < p < 0.1$) was observed in NS during hemorrhage, in consonance with the lower levels of Hb and HCT found during this period (Fig. 4 and Table 2). Blood calcium levels did not vary significantly over time in NS and S animals.

DISCUSSION

Although the study was not designed to precisely define outcome predictors, the data support the hypothesis that some systemic parameters can change in early HH. Animals subjected to a similar hemorrhage challenge and that were later separated into S and NS showed remarkably different responses to the early levels of blood loss. Survivors showed higher arterial blood pressure, ventilation, base excess and glucose extraction than NS. Plasma K^+ was lower in S during bleeding. Therefore, our data suggests that very early cardio-respiratory and metabolic compensations are essential for the survival of HH. These measurements possibly have prognostic value in shock since they are clinically available.

Protocol and data analysis

The stepwise hemorrhage protocol attempted to create a scenario where an initial rapid bleeding eventually slows down due to normal physiologic mechanisms, such as vasoconstriction, clot formation and progressively decreasing blood pressure¹⁵. Therefore, hemorrhage was studied as a non-steady-state condition that may represent a pre-hospital situation for cases of accidental hemorrhage, in particular in rural or military scenarios, where evacuation is delayed¹⁶. We are not aware of previous studies using this approach.

Since no resuscitation fluid was used, we investigated the blood biochemical changes without contaminating effects of these solutions⁹ and also eliminated other external factors such as changes in temperature and hemodilution effects leading to coagulopathies¹⁷. Because pre heparinization has protective effects to HH¹⁸, we studied only non-heparinized animals using a new double-lumen catheter technique for controlled bleeding and anticoagulant mixing¹⁹. We could not find other rat studies employing this technique for HH research. As recommended previously¹⁴, and used in an earlier study⁶, we selected a cut point leading to a 50 % mortality in order to produce a clinically relevant model and sufficient number of surviving animals to allow statistical comparisons between S and NS.

Cardiovascular and respiratory responses

Vasoconstriction is the normal immediate response to HH, but delayed hypotension (vasodilatory shock) may occur if the vascular smooth muscle fails to constrict. Since NS animals showed a lower MAP than S rats already during early blood withdrawal (Table 1) and a higher drop in hematocrit (hemodilution), our data are not consistent with an early failure to produce effective vasoconstriction²⁰, but may reflect differences in the cardiac performance between S and NS. The decline in cardiac function for NS can be partially explained by additional findings in this study such as increased potassium and decreased glucose, pH, TV and RR.

Respiratory compensation may account for the survival of the rats as shown in our previous study⁶. Our data indicate that early increased TV, leading to an increased minute volume, is essential to survival. The dissociation between RR and TV in early hemorrhage in relation to HH survival (Fig. 2) has not been previously reported, probably because most studies do not follow these parameters during bleeding. At non-lethal bleeding levels (25% and 35% of the total blood volume) tidal and minute volumes were already increased in S relative to NS. Our data suggest that the negative intrathoracic pressures (CVP in S is lower at 12 and 24 min than baseline measurements) induced by spontaneously increased in TV and MV generates an increase in the pressure gradient to improve venous return in an attempt to increase systemic pressures. This may be favorable to slowing or preventing progression to hemodynamic instability during conditions of central hypovolemia. Other authors observed very similar results using inspiratory impedance devices in humans^{21, 22}. The RR was decreased in nonsurvivors relative to survivors after HH was fully established, as also observed in our previous study⁶. Altogether, the data emphasize the importance of ventilation variables, typically not measured experimentally or clinically, and suggest an important role in the survival to HH⁶.

Reduction in blood volume during HH results in decreased cardiac output and decreased tissue O₂ delivery^{6, 23}. The latter may increase the activity of the anaerobic energy producing systems or decrease aerobic metabolism during HH, thus raising lactate and reducing plasma HCO₃⁻ concentrations²⁴. Blood oxygenation variables were always numerically lower in NS than in S. Progressively lower values of SO₂ during HH could be related to an effect of low arterial pH on Hb saturation, leading to a shift in the oxyhemoglobin dissociation curve to the right²⁵. The difference in MAP and in oxygenation variables between S and NS could favour the

idea of O₂ therapy in HH, possibly through the hyperoxic vasoconstriction that has been recently studied in detail by Atkins et al.²⁶. However, O₂ therapy to increase arterial PO₂ should be used with caution since hyperoxia may decrease functional capillary density²⁷ and has not improved outcome in some studies with rats²⁸.

The data from survivors are indicative of better ventilatory performance and consequently smaller tissue hypoxia in early stages of HH as shown by better acid-base behavior (Fig. 5, Table 3). Alfaro et al.²⁹ have also shown progressive changes in HCO₃⁻, BE and lactate in experimental graded hemorrhage up to 35%. Our metabolic findings confirm previous reports in humans^{8, 9, 30} and experimental animals³¹ including our own studies⁶.

Potassium and glucose

Changes in acid-base balance following HH affect K⁺ homeostasis since acidosis increases plasma K⁺³² via shifts between intracellular and extracellular compartments³³. Therefore, early derangements in the intracellular metabolic status³ and activation of K_{ATP} channels³⁴ could contribute to the low arterial pressure found in NS animals. Inhibition of K_{ATP} channels increased MAP and improved survival rate in rats³⁵.

Previous data⁶ suggest that the sudden death may be related to an acute toxic effect of high extracellular K⁺ in addition to progressive hypoxia, acidosis and hypoglycaemia, since similar levels of K⁺ are found at death in rats^{36–38}. We found a less pronounced hyperkalemia, but significantly different between S and NS (Fig. 5). The increase in extracellular K⁺ may be explained partially by the loss of hepatic K⁺³⁷ due to severe reduction in O₂ consumption in the liver³⁹ and large decreases in blood flow⁴⁰ during shock. Improved outcome has been shown in hypothermia^{19, 37, 41}, that also reduces hyperkalemia during HH.

Both S and NS showed hyperglycaemia during early hemorrhage, with significant hypoglycaemia in NS at later stages of HH. Hyperglycaemia results from various mechanisms, including decreased insulin production, increased catecholamine and glucose production, as well as insulin resistance. Hyperglycaemia has been also correlated with poor outcome in diverse studies^{42–44}. More importantly however, we found that the glucose extraction was higher in S than in NS (Fig. 6) during early bleeding. Therefore, our findings suggest that it is not only the hyperglycaemia, but the glucose extraction that may be important to survival. The role of glucose as an energy substrate to maintain homeostasis has been demonstrated^{23, 45}.

Limitations

Studies on early HH may not readily apply to hemorrhagic shock in patients, but our model reproduces the typical features of human HH. While controlled bleeding has been used to reproduce many features of severe hemorrhage in humans¹⁶, most studies do not investigate changes that take place during the blood loss as reported here. An important limitation of clinical studies is the relative inaccuracy to define the onset of shock, based on local scene or hospital presentation. These studies may include patients in shock for various periods of time. This limitation was not present in our study, although it is acknowledged that similar levels of bleeding may induce various degrees of HH. However, care must be taken when extrapolating the acid-base response of anesthetized animals to that expected in other experimental conditions, such as conscious animals during hemorrhage²⁹. In our model, maintaining a constant body temperature was attempted using a blanket. A certain degree of hypothermia often accompanies HH, and body temperature might interact with other mechanisms to differentiate variables that are likely to change in NS. Hence, it is plausible that maintenance of a constant body temperature may have masked some differences between S and NS, including the ability to maintain normal body temperature.

CONCLUSION

In summary, this study documents distinct patterns in S and NS soon after the onset of hemorrhage. The development of primary metabolic acidosis and further respiratory compensation was correlated with survivability: NS were not able to compensate for metabolic disturbances that started as early as 12 min after hemorrhage onset. Animals that died in less than 100 min (NS) showed worse cardiovascular, respiratory and metabolic responses than did S, including higher potassium, and lower blood oxygenation, MAP, TV and glucose extraction. The data suggest that some of the critical physiological responses leading to survival occur already during the hemorrhage period or at its early stages. Therefore, physiological responses that can differentiate between S and NS could be detected even before a lethal bleeding was reached.

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Figure 1.

Mean cumulative hemorrhaged volume expressed in ml per kg of body mass (left axis) and in percentage of estimated total blood volume (BV) (right axis), as a function of the mean time required to reach each hemorrhage level. Time = 0 min means the start of bleeding, followed by 3 pairs of data points, corresponding to each step of hemorrhage. Triangles = animals surviving less than 100 min of hemorrhage (nonsurvivors, n=22), O = rats surviving more than 100 min (survivors, n=22). All data points expressed as mean $\pm$ SEM (in most cases, the error is not visible because the error bar is smaller than the symbol).

Figure 2.

Respiratory rate, tidal volume and calculated minute volume for all studied animals. Data points shown at baseline (BL) are measurements performed before hemorrhage. Time = 0 min means the start of hemorrhage (first vertical dotted line). The time point when the target hemorrhaged volume was reached is marked by the second vertical dotted line.

† significantly different from baseline period; * significantly different from survivors at same time point. All data points expressed as mean; the bar above (or below) each data point is one SEM. All means after hemorrhage were significantly different from baseline.

Figure 3.

Oxygen content for arterial and venous blood and O₂ extraction ratio. O₂ extraction ratio was estimated as described in the methods section. Data shown as in Figure 2. † significantly different from baseline period; * significantly different from survivors at same time point.

Figure 4.

Systemic hematocrit for 22 nonsurvivors and 22 survivors. All means during and after HH were significantly different from baseline. Data shown as in Figure 2. † significantly different from baseline period; * significantly different from survivors at same time point.

Figure 5.

Arterial blood levels of standard base excess, lactate, HCO_3^- and potassium. All means after baseline were significantly different from baseline, except for the two measurements of potassium during hemorrhage for survivors. Data shown as in Figure 2.

Figure 6.

Arterial and venous plasma glucose levels, as well as calculated glucose extraction. Glucose extraction calculation was described in the methods section. Note blunted response of arterial glucose in nonsurvivors and of venous glucose in survivors, leading to major differences in glucose extraction. Data shown as in Figure 2. † significantly different from baseline period; * significantly different from survivors at same time point.

Table 1

Hemodynamic and cardiac parameters during and following hemorrhage.

Parameter	Group	Hemorrhage			Post-hemorrhage		
		Baseline	12 min	20 min	29 min	34 min	59 min
MAP (mmHg)	S	119.8 ± 4.0	70.7 ± 7.3 [†]	71.4 ± 6.2 [†]	58.4 ± 6.8 [†]	68.2 ± 7.0 [†]	70.8 ± 3.6 [†]
	NS	115.4 ± 3.0	53.3 ± 3.8 [†]	54.0 ± 3.7 ^{†*}	49.9 ± 4.0 [†]	52.6 ± 3.9 [†]	33.4 ± 3.2 [†]
SP (mmHg)	S	140.7 ± 6.2	95.2 ± 8.2 [†]	91.9 ± 6.8 [†]	85.5 ± 8.9 [†]	87.4 ± 7.4 [†]	88.4 ± 5.1 [†]
	NS	136.3 ± 2.5	72.8 ± 4.3 ^{†*}	74.0 ± 4.2 ^{†*}	72.6 ± 4.2 [†]	67.3 ± 5.0 ^{†*}	49.7 ± 4.7 ^{†*}
DP (mmHg)	S	99.5 ± 4.0	58.5 ± 7.1 [†]	58.9 ± 5.5 [†]	43.0 ± 5.1 [†]	51.2 ± 7.3 [†]	53.6 ± 3.4 [†]
	NS	95.0 ± 4.1	40.2 ± 4.7 [†]	42.0 ± 5.3 ^{†*}	37.8 ± 4.5 [†]	39.9 ± 4.0 [†]	25.6 ± 4.0 ^{†*}
PP (mmHg)	S	43.9 ± 4.1	37.4 ± 4.8	34.2 ± 4.7	39.0 ± 4.9	32.9 ± 5.4	32.0 ± 4.1
	NS	42.4 ± 3.5	33.3 ± 5.6	34.4 ± 5.5	34.7 ± 5.3	30.2 ± 3.6	28.7 ± 4.1

Values are means ± SEM from 17 survivors (S) and 20 nonsurvivors (NS).

MAP, mean arterial pressure; SP, systolic pressure; DP, diastolic pressure; PP, pulse pressure.

* significantly different from S group;

[†] significantly different from baseline period.

Table 2

Oxygenation parameters during and after hemorrhage.

Parameter	Group	Baseline		Hemorrhage		Post-hemorrhage		
		12 min	20 min	29 min	34 min	59 min		
aPO ₂ (mmHg)	S	92.1 ± 2.2	106.0 ± 3.0 [†]	110.4 ± 2.9 [†]	111.2 ± 2.9 [†]	111.5 ± 2.1 [†]	111.5 ± 1.9 [†]	
	NS	92.1 ± 1.6	110.6 ± 3.2 [†]	110.5 ± 3.3 [†]	114.3 ± 3.3 [†]	109.4 ± 2.6 [†]	105.8 ± 4.8 [†]	
vPO ₂ (mmHg)	S	47.4 ± 1.7	42.0 ± 3.5	36.1 ± 3.0 [†]	28.6 ± 1.6 [†]	28.1 ± 1.2 [†]	28.3 ± 1.3 [†]	
	NS	44.4 ± 1.4	28.6 ± 2.9 ^{†*}	24.5 ± 1.7 ^{†*}	29.2 ± 3.7 [†]	25.7 ± 2.4 [†]	21.5 ± 1.1 ^{†*}	
aSO ₂ (%)	S	95.3 ± 0.7	98.6 ± 0.6 [†]	99.2 ± 0.5 [†]	99.2 ± 0.4 [†]	97.8 ± 0.5 [†]	97.7 ± 0.4 [†]	
	NS	95.0 ± 0.8	99.4 ± 0.4 [†]	99.0 ± 0.8 [†]	98.8 ± 0.6 [†]	91.8 ± 2.5	92.2 ± 2.1	
vSO ₂ (%)	S	62.0 ± 2.4	50.1 ± 5.4 [†]	36.3 ± 4.1 [†]	24.9 ± 4.1 [†]	24.3 ± 2.7 [†]	19.4 ± 2.2 [†]	
	NS	61.5 ± 1.7	39.9 ± 5.2 [†]	22.3 ± 2.5 ^{†*}	17.3 ± 3.1 [†]	14.6 ± 1.6 ^{†*}	11.6 ± 2.3 ^{†*}	
aHb (g dl ⁻¹)	S	13.1 ± 0.2	11.5 ± 0.4 [†]	10.9 ± 0.3 [†]	9.9 ± 0.3 [†]	9.8 ± 0.2 [†]	9.8 ± 0.3 [†]	
	NS	13.0 ± 0.3	10.2 ± 0.6 ^{†*}	9.4 ± 0.6 ^{†*}	8.6 ± 0.3 ^{†*}	10.3 ± 0.5 [†]	10.3 ± 0.6 [†]	
vHb (g dl ⁻¹)	S	13.1 ± 0.2	11.5 ± 0.4 [†]	10.9 ± 0.3 [†]	9.9 ± 0.3 [†]	9.8 ± 0.2 [†]	9.8 ± 0.3 [†]	
	NS	13.2 ± 0.3	10.2 ± 0.6 ^{†*}	9.4 ± 0.6 ^{†*}	8.6 ± 0.3 ^{†*}	10.3 ± 0.5 [†]	10.3 ± 0.6 [†]	

Values are means ± SEM from 22 survivors (S) and 22 nonsurvivors (NS).

a, arterial; v, venous; PO₂, venous partial pressure of oxygen; SO₂, hemoglobin oxygen saturation; Hb, hemoglobin concentration.

* significantly different from S group;

† significantly different from baseline period.

Table 3

Arterial blood biochemical parameters during and after hemorrhage.

Parameter	Group	Hemorrhage			Post-hemorrhage		
		Baseline	12 min	20 min	29 min	34 min	59 min
PCO ₂ (mmHg)	S	39.6 ± 1.2	31.6 ± 2.2 [†]	28.1 ± 1.9 [†]	26.1 ± 1.5 [†]	26.9 ± 1.5 [†]	26.2 ± 1.6 [†]
		38.6	27.7	25.5	23.2	21.4	20.6
	NS	± 1.2	± 2.2 [†]	± 3.0 [†]	± 2.2 [†]	± 1.5 ^{†*}	± 1.3 ^{†*}
		7.436	7.468	7.476	7.448	7.413	7.402
pH	S	± 0.01	± 0.02 [†]	± 0.02 [†]	± 0.02	± 0.02	± 0.02
		7.441	7.475	7.430	7.403	7.326	7.177
	NS	± 0.01	± 0.02	± 0.03	± 0.03	± 0.03 ^{†*}	0.04 ^{†*}
		135.5	130.5	132.3	130.4	132.0	132.3
Na ⁺ (mmol l ⁻¹)	S	± 1.6	± 3.0	± 1.1	± 2.4 [†]	± 1.6	± 1.7
		134.0	125.1	129.8	127.8	129.4	135.5
	NS	± 1.6	± 4.0 [†]	± 2.4	± 2.9 [†]	± 2.2	± 2.1
		1.09	1.03	0.99	1.01	1.04	1.09
Ca ⁺⁺ (mmol l ⁻¹)	S	± 0.04	± 0.05	± 0.06	± 0.03	± 0.07	± 0.04
		1.02	1.08	1.03	1.02	0.97	0.99
	NS	± 0.05	± 0.05	± 0.04	± 0.02	± 0.08	± 0.08

Values are means ± SEM from 22 survivors (S) and 22 nonsurvivors (NS).

PCO₂, partial pressure of CO₂.

* significantly different from S group;

[†] significantly different from baseline period.