CARDIAC PERFORMANCE DURING ACUTE HEMORRHAGIC SHOCK IN PENTOBARBITAL-ANESTHETIZED HAMSTERS USING LEFT VENTRICULAR PRESSURE-VOLUME MEASUREMENT

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ABSTRACT
Hemorrhagic shock results from massive blood loss, leading to hypotension, ischemia, heart failure and multi-organ dysfunction. The analysis of cardiac function in vivo is complex and even more challenging during hemorrhagic shock, especially in small animals. Cardiac function measurements require animals to be under anesthesia. Sodium pentobarbital is a common general anesthetic used for cardiovascular physiological studies in experimental animals. This study was designed to evaluate cardiac function during an acute hemorrhagic shock in pentobarbital-anesthetized hamsters. Animals were subjected to a hemorrhage of 40% of blood volume. Cardiac performance was evaluated using a miniaturized pressure-volume conductance catheter. One group of animals was under anesthetic without hemorrhage as a control group and other group was under anesthetic with hemorrhagic shock. We found that dP/dt max was 77% and 68% of baseline in control and hemorrhagic shock groups, respectively. An estimated left ventricular end-systolic elastance in a hemorrhagic shock group was higher compared with a control group. Cardiac output, stroke volume and stroke work profoundly decreased after hemorrhage. Animals anesthetized with sodium pentobarbital evidently had depressive systolic cardiac function. The depression was more profound in systolic and diastolic cardiac function during hemorrhagic shock.

KEY WORDS
cardiac function, pentobarbital, hemorrhage, shock

1. Introduction
Hemorrhagic shock is a pathological state with insufficient blood volume and low arterial blood pressure in a circulatory network, leading to hypoperfusion, low oxygen delivery and anaerobic metabolism. Hemorrhagic shock may effectively affect the pumping performance of the heart and finally cause cardiac dysfunction. To measure and assess cardiac function, animals need to be maintained under anesthesia. Most anesthetics are cardio-depressive agents which have significant effects on cardiovascular activities [1-3]. Sodium pentobarbital is a common general anesthetic using in experimental animals for cardiovascular physiological study. Without shock condition, cardiac function was altered and decreased with sodium pentobarbital [4-6].

This study was designed to evaluate cardiac function during an acute hemorrhagic shock in pentobarbital-anesthetized hamsters. We applied a miniaturized conductance catheter to measure left ventricular pressure and volume in the fixed volume (40% of blood volume) hemorrhagic shock protocol. The indices of systolic and diastolic function were derived from the left ventricular pressure-volume measurements to quantify and evaluate cardiac performance between with and without hemorrhagic shock conditions.

2. Materials and Methods

2.1 Animal preparation
Anesthetized male Golden Syrian hamsters (Charles River Laboratories, Boston, MA) weighing 60-70g were used in this study. The NIH Guide for Care and Use of Laboratory Animals was followed for animal handling and care. The experimental protocol was passed the approval of institutional animal care committee. Sodium pentobarbital (50mg/kg, i.p.) was used as a general anesthetic to perform an animal surgery. The left jugular vein was catheterized for fluid infusion and left femoral artery was cannulated for blood pressure measurement and blood withdrawal. Animals were performed tracheotomy and cannulated with a polyethylene-90 tube to facilitate animals’ breathing. Animals were maintained the body temperature by putting in a supine position on a heating pad. During experiment, a small bolus of sodium pentobarbital (10-15mg/kg, i.p.) will be administered if animal responses to a toe pinching.
2.2 Inclusion criteria

Animals under anesthesia were included in the study if animals had no surgical blood loss and systemic parameters were within the normal range by i) mean arterial blood pressure (MAP) > 80 mmHg, ii) heart rate (HR) > 320 beats/minute and iii) systemic hematocrit (Hct) > 45%.

2.3 Systemic parameters

MAP and HR were monitored continuously throughout the experiment (MP150, Biopac System Inc., Santa Barbara, CA), except when blood was sampled for laboratory parameters and blood conductance calibration and withdrawn for hemorrhage. The Hct was determined from centrifuged arterial blood samples taken in heparinized capillary tubes. Hemoglobin (Hb) content in the blood sample was measured by spectro-photometer (B-Hemoglobin, Hemocue, Stockholm, Sweden). Both Hct and Hb content were measured at baseline and the end of experiment (90min after beginning of shock).

2.4 Cardiac function

Cardiac function was evaluated via the closed chest method. A 1.4F pressure-volume conductance catheter (PV catheter; SPR-839, Millar Instruments, TX) was inserted into the right common carotid artery and, then, was advanced passing through the aortic valve into the left ventricle [6]. Parallel volumes (Vp) at the baseline, 15 min after hemorrhage and the end of shock period were determined by a small bolus intravenous injection of 15% hypertonic saline (10μl). The Vp during the shock period was assumed to be similar to that determined at the end of shock period. The left ventricular pressure and volume measurements were instantaneously digitized and acquired (MPVS300, Millar Instruments, TX and PowerLab 8/30, ADInstruments, CO).

2.5 Estimation of left ventricular blood volume

To determine blood volume in the left ventricle, it is necessary to convert the measured blood conductance (RVU: relative volume unit) to the actual blood volume (μl) as described by Chatpun and Cabrales [7].

2.6 Estimation of end-systolic elastance

The left ventricular end-systolic elastance (Ees) was estimated using the bilinearly approximated time-varying elastance curve from the selected single beat [8]. This approximation basically considered pressure values, systolic time interval, end-systolic volume and stroke volume during isovolumic contraction and ejection phases.

2.7 Acute hemorrhagic shock protocol

Anesthetized animals were withdrawn 40% of estimated blood volume (BV) via the femoral artery catheter within 15 minutes to induce an acute hemorrhage. The total BV was estimated as 7% of body weight. The shock condition was conducted for 90 minutes. Systemic parameters (MAP and HR) were analyzed at the interested time points as schematically shown in Figure 1. Animals were maintained under anesthesia over the time of experiment.

2.8 Experimental groups

Animals were randomly assigned into two groups: a group without hemorrhagic shock as a control group and a group with hemorrhagic shock. There were 5 animals in each group (n=5).

2.9 Data analysis

Cardiac function data were analyzed with PVAN software (version3.6, Millar Instruments, TX). Indices of systolic and diastolic function were calculated including maximum rate of pressure change (dP/dtmax), ejection fraction (EF), left ventricular end-systolic pressure (Pes), minimum rate of pressure change (dP/dtmin), ratio between dP/dtmax and end-diastolic volume (dP/dtmax/Ved), left ventricular relaxation time constant (Tau), maximum filling volume rate (dV/dtmax) and left ventricular end-diastolic pressure (Ped). Other cardiac function indices such as cardiac output (CO), stroke work (SW) and stroke volume (SV) were also considered. In addition, stroke work was normalized by stroke volume (SW/SV), representing the work done by the heart per unit volume. The values from selected 8-12 cardiac cycles at each time point were averaged for cardiac function indices. These indices were analyzed before hemorrhage (baseline) and after hemorrhage (30, 45, 60 and 90 min after beginning of shock).

2.10 Statistical analysis

Results are presented as mean ± standard deviation (SD). The values are mostly presented as relative to level at baseline. A ratio of 1.0 defines no change from baseline, whereas lower or higher ratios represent the changes proportionally lower or higher compared to baseline. Data between interested time points within a group were analyzed with an analysis of variance for repeated measures (ANOVA) followed by post hoc analyses with the Tukey’s multiple comparison tests. Two-way ANOVA test with a post hoc t-test with Bonferroni correction for multiple comparisons was used to compare between groups at time point of interest. All statistics were analyzed using GraphPad Prism 4.01 (GraphPad Software, San Diego, CA). A p<0.05 indicated a statistical significance.
3. Results

3.1 Systemic parameters

Table 1 presents a significantly decreased on Hct and Hb in the hemorrhage group compared with the control group (p<0.05).

MAP showed a significant decrease after hemorrhage compared to baseline as presented in Figure 2 (p<0.05). During the early phase of shock (from SH0 to SH30), MAP continually increased and gradually dropped over the time until the end of observation. At the end of experiment, MAP was about 50% of baseline and animals still survived in the hemorrhage group. Interestingly, MAP in the control group also gradually decreased over the observation period. The MAP in the control group at the end of experiment was about 80% of baseline and significantly dropped relative to baseline (p<0.05). The significant difference between groups in MAP was observed over the shock period (p<0.05). On the other hand, there was only significant difference between groups in HR at 60 min after hemorrhage. However, HR in the control group slightly increased but HR in the hemorrhage group gradually decreased over the time as shown in Figure 3.

Table 1. Laboratory parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>90 min shock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct, %</td>
<td>Control</td>
<td>Hemorrhage</td>
</tr>
<tr>
<td></td>
<td>53 (2)</td>
<td>49 (3)</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>16.0 (0.8)</td>
<td>14.7 (1.3)</td>
</tr>
</tbody>
</table>

Values are means (SD). † p<0.05 compared between groups.

3.2 Left ventricular systolic indices

The dP/dt\textsubscript{max}, the load-dependent contractility index, in both groups gradually decreased over the observation time in a similar direction as illustrated in Figure 4A. At the end of experiment (SH90), dP/dt\textsubscript{max} was 77% and 68% of baseline in the control and hemorrhage groups, respectively. However, there was not significantly different in dP/dt\textsubscript{max} between the control and hemorrhage groups. In the control group, EF did not change from the baseline level, while it was higher than baseline level in the hemorrhage group (Figure 4B). Although there was a difference in EF between two groups, it was not statistically significant. At the late phases of shock (SH60 and SH90), the decrease of end-systolic pressure (P\textsubscript{es}) in the control group was significantly pronounced relative to baseline as presented in Figure 4C (p<0.05). On the other hand, P\textsubscript{es} in the hemorrhage group did not significantly change relative to baseline. Figure 4D presents that the ratio of dP/dt\textsubscript{max} and V\textsubscript{es} in the hemorrhage group dropped after SH30 to the end of experiment but it was still higher than baseline. In contrast, this ratio in the control group was lower than baseline and it significantly decreased relative to baseline at the end of shock period (p<0.05).

The dP/dt\textsubscript{max}/V\textsubscript{es} only showed a significant difference between groups at 30min after hemorrhage (p<0.05).
time constant (Tau) than animals without hemorrhagic shock due to less blood volume. The $dV/dt_{max}$ was also derived to evaluate the diastolic function of the heart. Regarding to a reduction in blood volume, $dV/dt_{max}$ in the hemorrhage group dropped significantly compared with baseline (Figure 7C; $p<0.05$). No statistically significant change in $P_{ed}$ relative to baseline was found in both the control and hemorrhage groups as well as no significance between groups in $P_{ed}$ after hemorrhage (Figure 7D).

3.3 Left ventricular end-systolic elastance

Figure 5 presents the approximated left ventricular end-systolic elastance ($E_{es}$) during hemorrhagic shock. Animals in the control group showed a significant decrease in $E_{es}$ relative to baseline over the experimental period ($p<0.05$). The significant difference between groups was found at 30 and 60 min after hemorrhage (SH30 and SH60, $p<0.05$). Figure 6 shows the linear regression between $dP/dt_{max}/V_{ed}$ and $E_{es}$ for both the control and hemorrhage groups. It was found that $dP/dt_{max}/V_{ed}$ had a linear relationship with $E_{es}$ ($E_{es}=0.83 dP/dt_{max}/V_{ed} + 0.18$, $r=0.55$ for the control group and $E_{es}=0.55 dP/dt_{max}/V_{ed} + 0.28$, $r=0.69$ for the hemorrhage group).

Figure 7 presents the approximated left ventricular end-systolic elastance ($E_{es}$) during hemorrhagic shock. Broken line represents the baseline level. Values are presented as means ± SD. * $p<0.05$ compared with baseline. † $p<0.05$ compared between groups.

3.4 Left ventricular diastolic indices

The $dP/dt_{min}$ significantly decreased from baseline over the time in the hemorrhage group as shown in Figure 7A ($p<0.05$). In contrast, animals in the control group maintained $dP/dt_{min}$ at the baseline level. However, $dP/dt_{min}$ was significantly different between groups over the shock period ($p<0.05$). Figure 7B clearly shows that animals under hemorrhagic shock had a higher relaxation constant (Tau) than animals without hemorrhagic shock due to less blood volume. The $dV/dt_{max}$ was also derived to evaluate the diastolic function of the heart. Regarding to a reduction in blood volume, $dV/dt_{max}$ in the hemorrhage group dropped significantly compared with baseline (Figure 7C; $p<0.05$). No statistically significant change in $P_{ed}$ relative to baseline was found in both the control and hemorrhage groups as well as no significance between groups in $P_{ed}$ after hemorrhage (Figure 7D).

Figure 4. Left ventricular systolic function indices derived by PV conductance catheter at baseline and during hemorrhagic shock. (A) Maximum rate of pressure change ($dP/dt_{max}$). (B) Ejection fraction (EF). (C) Left ventricular end-systolic pressure ($P_{es}$). (D) Ratio of maximum rate of pressure change to end-diastolic volume ($dP/dt_{max}/V_{ed}$). Broken line represents the baseline level. Values are presented as means ± SD. * $p<0.05$ compared with baseline. † $p<0.05$ compared between groups.

Figure 6. Linear regression between $dP/dt_{max}/V_{ed}$ and the estimated end-systolic elastance ($E_{es}$) with 95% confidence of interval.

Figure 7. Left ventricular diastolic function indices derived by PV conductance catheter at baseline and during hemorrhagic shock. (A) Minimum rate of pressure change ($dP/dt_{min}$). (B) Relaxation time constant (Tau). (C) Maximum filling volume rate ($dV/dt_{max}$). (D) Left ventricular end-diastolic pressure ($P_{ed}$). Broken line represents the baseline level. Values are presented as means ± SD. * $p<0.05$ compared with baseline. † $p<0.05$ compared between groups.

Figure 8. Other left ventricular cardiac function indices derived by PV conductance catheter during hemorrhagic shock. (A) Cardiac output (CO). (B) Stroke work (SW). (C) Stroke volume (SV). (D) Work done per stroke volume (SW/SV). Broken line represents the baseline level. Values are presented as means ± SD. * $p<0.05$ compared with baseline. † $p<0.05$ compared between groups.
3.5 Cardiac function parameters

Figure 8A shows the CO during hemorrhagic shock. Animals in the control group maintained CO slightly higher than the baseline level for the entire observation time. On the other hand, animals in the hemorrhage group had a significantly lower CO relative to baseline and the control group (p<0.05). The CO in the hemorrhage group essentially dropped to 60% of baseline during a shock period.

The SW in the control group was lower than the baseline level during a shock phase as demonstrated in Figure 8B. As expected, animals in the hemorrhage group showed a significant decrease in SW as a result of blood loss (p<0.05). Similarly to SW, the significant reduction in SV was caused by blood loss during hemorrhage (Figure 8C; p<0.05). However, when we considered SW/SV, it was interesting that this ratio was maintained over the time of observation in the control group but not in the hemorrhage group (Figure 8D). Despite the reduction of SW/SV in the hemorrhage group, there was no significant difference in SW/SV comparing between two groups.

4. Discussions

We found that sodium pentobarbital (PB) significantly decreased systolic cardiac performance but did not markedly affect on diastolic function in animals without hemorrhage. Interestingly, pentobarbital-anesthetized animals with hemorrhagic shock had a profound depression in diastolic function beyond the effects observed in anesthetized animals without hemorrhage. The cardiac contractility was enhanced during the early stage of hemorrhagic shock as a result in an increase in $E_{es}$ and $dP/dt_{max}/V_{ed}$. Our results further suggest that hemorrhagic shock effectively caused a reduction in load-dependent parameters such as CO, SW, SV and $dP/dt_{max}$.

PB is a cardiodepressive agent and has negative effects on the heart [6, 9, 10]. Our data in animals without hemorrhage shock agree with Manders and Vatner’s work that PB decreased cardiac contractility and slightly altered cardiac output [11]. However, we found that PB progressively decreased MAP which is contradicted with their results. Yang et al. used an echocardiography to assess cardiac function in both conscious and anesthetized mice [5]. They reported that PB significantly altered cardiac hemodynamics and performance in mice, probably as the results of sympathetic activity inhibition and depression in myocardial contractility. Recently, the study about the effects of anesthetic agents on left ventricular function in mice and rats using PV conductance catheter has shown that PB caused the indices of systolic and diastolic function much lower than other anesthetics i.e. isoflurane and mixture of ketamine and xylazine [6]. However, our results suggest that PB majority impaired systolic and contractile function but slightly altered diastolic function in the hamster model. The depression in systolic function may be a result of suppression of plasma catecholamine (epinephrine and norepinephrine) concentration by PB [2, 12]. Furthermore, PB inhibits the reflex activation of the sympathetic nervous system and causes hypothermia in animals [12, 13]. PB also produces respiratory depression which can attenuate the gas exchange activity [14]. Conclusively, PB plays an important role in the reduction of cardiac performance and compensatory responses.

When we considered the indices of diastolic function between the control and hemorrhage groups, we found that the depression of diastolic function was strongly caused by the consequences of hemorrhage, not by the effects of PB.

Hemorrhagic shock progressively causes hypotension, hypothermia and a reduction of oxygen delivery in consequences. These consequences significantly impair cardiac performance in the severe hemorrhagic shock. However, the degree of hemorrhagic shock depends on the amount of blood loss and the duration of shock [15]. Using fixed volume hemorrhagic shock in our study, $dP/dt_{max}$ gradually decreased during the shock period in the hemorrhage group, as same as that was observed over time in the control group. This finding may imply that the effects of PB still dominated in the hemorrhage group. Other load-dependent systolic function index such as ejection fraction (EF) during the shock period was higher than the baseline which was controversial with $dP/dt_{max}$. However, our results were in agreement with the work of Welte et al. [16]. Their study measured hemodynamic and cardiac contractility in anesthetized pigs under hypovolemic shock condition and showed that $dP/dt_{max}$ decreased but EF increased at shock stage relative to baseline. There were several studies suggested that the cardiac contractility was increased during the shock as a result of autonomic response to the hypotension [17, 18]. Our results showed that other indices of systolic function such as $P_{es}$, $dP/dt_{max}/V_{ed}$ and approximated $E_{es}$ apparently were increased during the shock period and higher than the baseline, implying the cardiac contractile function was enhanced due to the compensatory mechanism. However, there was a study demonstrated that cardiac contractility was depressed during hemorrhagic shock [15]. Theoretically, myocardial contractility should be independent of preload, afterload and heart rate thus it is difficult to measure. Therefore, it is still controversial and the interpretation of cardiac contractility depends on the measurement techniques and animal models.

We also found that diastolic function of the heart was effectively depressed during the shock stage as demonstrated by the significant decrease in $dP/dt_{max}$ and $dV/dt_{max}$. The relaxation time constant was significantly higher than baseline after hemorrhage, showing the depletion in the isovolumic relaxation phase. As the reduction of $dV/dt_{max}$ during the shock stage, it indicated that the vascular return or preload was attenuated and the filling phase was delayed due to blood loss.
The load dependent cardiac parameters such as SW and SV significantly decreased during the hemorrhagic shock. However, the work done per stroke volume (SW/SV) during the shock stage was maintained. This possibly indicates that the rate of energy consumption per unit volume was maintained to perform cardiac activities even though hemorrhagic shock existed.

By this study, several concerns should be considered. First, the orientation and position of PV conductance catheter in the left ventricle can affect the accuracy of left ventricular volume. Second, the parallel volume may vary during hemorrhagic shock and play a role in the calculated parameters such as EF, Ees and dP/dt max/V ed. Third, the body core temperature of animals also influences the myocardial electrical conductivity and cardiac chronotropy.

5. Conclusion

In summary, our study shows that sodium pentobarbital and hemorrhage significantly affect both systolic and diastolic cardiac function. The roles of both sodium pentobarbital and hemorrhagic shock on the heart performance are presented as a diagram in Figure 9. Sodium pentobarbital inhibits sympathetic nervous system, leads to hypothermia, depresses respiratory system and attenuates cardiac performance. In addition, hemorrhagic shock impaired oxygen delivery by reducing an amount of red blood cells and induced a release of myocardial depressive factors. Therefore, when animals anesthetized with sodium pentobarbital were under hemorrhagic shock, cardiac function was profoundly depressive.

Acknowledgements

The authors thank Cynthia Walser for the surgical preparation of the animals. This work was partially supported by Bioengineering Research Partnership grant R24-HL64395, Program project P01-HL071064 and grants R01-HL62354.

References


