Continuous peripheral resistance measurement during hemorrhagic hypotension

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Submitted 24 February 2004; accepted in final form 18 June 2004


In most hemorrhagic shock studies, variables of interest are sampled at fixed intervals while the mean arterial pressure (MAP) is kept at a low but constant level. However, because the time course of physiological responses of different animals may not be synchronized, composite data collected at fixed time intervals may not accurately reflect the sequence of events. Poor resolution of the time course may result in investigators missing both the timing and size of maximal changes (3). The evaluation of variables that may change rapidly over time can be even more affected by this sampling effect. Few studies provide continuous evaluation of total peripheral resistance (TPR) mainly because cardiac output (CO) is usually measured using dilution or microsphere tech-

Received for publication July 15, 2004; accepted in final form June 18, 2004.

Torres, Luciana N., Ivo P. Torres Filho, R. Wayne Barbee, M. Hakam Tiba, Kevin R. Ward, and Roland N. Pittman. Continuous peripheral resistance measurement during hemorrhagic hypotension. Am J Physiol Heart Circ Physiol 287: H2341–H2345, 2004. First published July 15, 2004; doi:10.1152/ajpheart.00179.2004.—We tested the hypotheses that continuous total peripheral resistance (TPR) measurements are superior to intermittent data collection and that variables related to TPR can be used to distinguish between survivors and nonsurvivors (NS), respectively, of prolonged hemorrhagic hypotension (HH). One week after a transit-time ultrasound probe was implanted on their ascending aorta, 21 rats were subjected to 4 h of HH at 40 mmHg. Measurements were made before and up to 4 h after initiation of HH. Additional bleeding or Ringer-lactate (RL) infusion was used to maintain HH. TPR was continuously measured online using recordings of blood flow and arterial pressure. Approximately 67% of the rats survived ≥3 h; others were considered NS. Data collected at 30-min intervals failed to detect the maximum value of TPR (TPRmax). The times to reach TPRmax were similar for survivors and NS and were strongly correlated with the bleeding end points and with the RL infusion-onset times. However, survivors showed higher TPRmax values than NS (P < 0.005) and had a significantly longer period than NS during which TPR was above baseline level (116 ± 20 vs. 51 ± 10 min). In conclusion, 1) the transit-time ultrasound technique at high sampling rate allowed continuous and accurate real-time monitoring of TPR, 2) the bleeding end point and RL infusion-onset times may be used as surrogates of the time to TPRmax, 3) TPRmax of survivors and NS could be detected only using a continuous TPR measurement, and 4) differences between survivors and NS could be revealed by the continuous TPR curve.

hemorrhage; blood pressure; total peripheral resistance; cardiac output; transit-time ultrasound

MATERIALS AND METHODS

This study conforms to the Public Health Service Policy on Human Care and Use of Laboratory Animals (August 2002) and the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.” Twenty-one male Sprague-Dawley rats (Harlan; Indianapolis, IN) that weighed 281 ± 9 g (means ± SE) were used in the study.

Aortic flow probe implantation. Rats were anesthetized with 3% isoflurane-97% O2 (Abbott Laboratories; North Chicago, IL) and then intubated using a polyethylene tube connected to a ventilator (Kent Scientific; Torrington, CT). A lateral thoracotomy was performed under aseptic conditions. A Silastic tube connected to a syringe was inserted into the chest cavity through a separate opening. A transverse ultrasonic flow probe (model 2.5SB; Transonic Systems; Ithaca, NY) was positioned around the isolated ascending aorta, and the chest cavity was closed. The thoracic negative pressure was restored by...
simultaneously expanding the lungs and evacuating the air inside the thorax using the Silastic tube. The tube was then removed. The probe cable was tunneled under the skin to the nape of the neck where it was then sutured.

**Systemic measurements.** After a 6–9-day recovery period, the animals were subjected to tracheostomy and catheterizations as described previously (17). In brief, rats were anesthetized with a mixture of ketamine (70 mg/kg ip; Fort Dodge Animal Health; Fort Dodge, IA) and acepromazine (3 mg/kg ip; Vedco; St. Joseph, MO) and received subsequent constant infusion (0.24–0.36 mg/kg/h iv) of alfaxonol/alfadolone acetate (Saffan; Schering-Plough Animal Health; Welwyn Garden City, UK). A femoral artery was cannulated for fluid withdrawal using a syringe pump (model PHD2000; Harvard Apparatus; Holliston, MA). The carotid artery and the jugular vein were cannulated and connected to pressure transducers to continuously measure arterial pressure and central venous blood pressure, respectively. All catheters were flushed as needed with heparinized saline. The core temperature was maintained at 35.5–37.0°C. Blood analyses were performed in paired arterial and venous samples (0.1 ml each) collected at various time points using heparinized glass capillaries, a blood gas analyzer, and a CO oximeter (ABL 725 and OSM3; Radiometer; Copenhagen, Denmark). All blood samples were immediately replaced by equal volumes of Ringer I- lactate (RL; Baxter; Deerfield, IL).

**Protocol.** Animals were positioned in the left lateral decubitus position and were heparinized (260 U/kg of body wt). After at least a 20-min stabilization period, baseline measurements were made. Hypotension was then induced by slow removal (0.3–0.5 ml/min) of blood over 15–20 min until MAP reached 40 mmHg. Additional blood was withdrawn as needed to lower MAP to 40 mmHg. RL infusion was used to maintain MAP if it decreased below 40 mmHg. During each blood withdrawal or RL infusion, the times and volumes were noted. Systemic blood gases and metabolic variables along with systemic hemodynamics were measured when the 40 mmHg target was reached, which was considered to be time point t = 0, and at 0.5, 1, 2, 3, and 4 h later. Control animals (n = 4) were subjected to all surgical and experimental procedures described above except for the hemorrhage.

**Data acquisition and analysis.** The amplified outputs from the pressure transducers (DA100C; Biopac Systems; Goleta, CA) and the aortic flowmeter were connected to a computer for continuous online data acquisition at a rate of 500 Hz (Acqknowledge 3.7.2 software and MP150 hardware; Biopac Systems). Mean values were continuously computed (also at 500 Hz) from the pulsatile signals on a cycle-by-cycle basis. Mean CO was estimated from the aortic flow, and MAP was estimated from the arterial pressure. TPR was continuously calculated as MAP/CO (expressed as mmHg·ml⁻¹·min⁻¹). The TPR was normalized by the average value during the baseline period (TPR₀), and this value was used to calculate the TPR as a percentage change from the baseline (TPR%) using the equation TPR% = (TPR − TPR₀)/TPR₀. TPRmax was defined for each animal as the maximum TPR value found during the hypotensive period. If more than one maximum value was found, only the last value was considered for subsequent analyses of that experiment. Values of TPR at specific points (TPR at 30 min, for instance) were computed as 1-min averages.

**Statistics.** Data are expressed as means ± SE. Absolute values were analyzed using two-way ANOVA with repeated measures. When a significant F value was encountered, post hoc analyses were performed between groups with Bonferroni’s correction for multiple comparisons. Differences between survivor and NS for TPRmax (in percent change from baseline) were analyzed using unpaired t-test because these data did pass the normality and equal variance tests as performed using SigmaStat statistical software (Jandel Scientific). For correlation analysis, linear least-squares regressions were performed, and significance of the correlation coefficients was tested. The P values correspond to two-tailed tests with significance set at 0.05.

**RESULTS**

Control animals, which were subjected to all procedures except HH, showed stable physiological variables throughout the experimental period of 5 h. Based on a mortality rate of 50% as recommended previously (7), a cutting point was established before the execution of the experiments. A 3-h time point was selected to divide survivors and NS because nearly equal numbers of survivors and NS were obtained using this cutoff point. To avoid any bias, this was done before the analysis was performed. Animals subjected to HH were divided into two groups: 9 rats survived 3–4 h (mean survival time, 219.5 ± 11.8 min), and 8 animals died in <3 h after MAP reached 40 mmHg (mean survival time, 128.9 ± 12.1 min). The peak shed blood volumes, which were defined as the maximum blood volumes removed, were similar for survivors and NS (8.0 ± 0.4 and 8.8 ± 0.9 ml, respectively). The times from the onset of hemorrhage until a MAP of 40 mmHg was reached were also similar for all animals (average, 17.9 ± 1.2 min). The continuous measurement of CO and MAP allowed online monitoring of TPR throughout the experiment. Figure 1 shows examples of TPR tracings for a survivor and a nonsurvivor during the course of the experiment.

The continuous TPR curve from each animal was subjected to quantitative analysis by first labeling certain time points, which are indicated by letters in Fig. 1. On the basis of these points, the durations of some events were computed and are presented in Table 1. Distantly spaced time-based measurements of TPR can be identified in Fig. 1 by the intersections between each curve and the vertical dotted lines. Intervals of 30 min were chosen because these represent sampling intervals that are frequently used in experiments lasting several hours. Figure 1 illustrates that the time of occurrence and the correct value of TPRmax from both survivors and NS would have been missed if data were collected at 30-min intervals. Similar findings were obtained for most of the other recordings. The TPRmax value estimated from data collected each 30 min was.
TPR IN HEMORRHAGIC HYPOTENSION

Table 1. Time intervals of continuous TPR recordings during hemorrhagic hypotension

<table>
<thead>
<tr>
<th>Time Interval Description</th>
<th>Survivors</th>
<th>Nonsurvivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>From 40 mmHg to TPR max (point B to C)</td>
<td>21.2±6.8</td>
<td>15.6±3.8</td>
</tr>
<tr>
<td>From 40 mmHg to baseline (point B to D)</td>
<td>116.3±20.1</td>
<td>50.8±9.5*</td>
</tr>
<tr>
<td>TPR above baseline (point A to D)</td>
<td>134.6±20.4</td>
<td>68.2±10.1*</td>
</tr>
<tr>
<td>TPR below baseline (point D to E)</td>
<td>102.9±11.1</td>
<td>78.0±7.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9 rats (survivors) and 8 rats (nonsurvivors). Points A–E are described in Fig. 1. TPR max, maximum total peripheral resistance. *P < 0.05, significantly different from survivors.

significantly smaller than that estimated from data collected using continuous TPR recordings.

Analyzing the time interval from the time that 40 mmHg was reached until the time that TPR max was achieved (points B to C in Table 1 and Fig. 1), no significant differences between survivors and NS were found, although the average TPR max value was significantly higher in survivors than in NS (66.2 ± 11.0 and 29.8 ± 6.5%, respectively). The time period from TPR at 40 mmHg of MAP to the baseline level of TPR (points B to D in Table 1 and Fig. 1) was computed, and this interval was significantly longer for survivors than NS. The amounts of time that TPR remained above (points A to D in Table 1 and Fig. 1) and below (points D to E in Table 1 and Fig. 1) baseline levels were also longer for survivors than NS.

The removed and infused volumes during HH are plotted in Fig. 2 with the continuous TPR curves for one nonsurvivor and one survivor. The highest value of TPR for both rats occurred between the end of the hemorrhage and the start of the crystalloid infusion. TPR max was achieved at 16 and 14 min after 40 mmHg was reached for the survivor and nonsurvivor, respectively.

The average time from MAP at 40 mmHg (t = 0 min) until the end of hemorrhage (bleeding end point) was not significantly different between NS and survivors (average, 15.2 ± 2.9 min). The average time from MAP at 40 mmHg until the start of RL infusion was also statistically similar for all animals (24.2 ± 3.8 min). TPR max was reached at an average time of 18.6 ± 4.0 min, and this time was not significantly different from either the bleeding end point or the RL-infusion onset. Figure 3 illustrates the relationship between the time to reach TPR max and the bleeding end point or the time that RL infusion started for both groups of animals. Linear, positive, and significant correlations were found for both relationships. Furthermore, the identity line is situated within the 95% confidence interval of each one of the linear regression lines, which further suggests that TPR max was reached between the end of hemorrhage and the start of RL infusion.

DISCUSSION

The balance between vasodilator and vasoconstrictor influences may be the key factor that determines survival (2). Our data showed a prolonged constriction with higher TPR values, especially for survivors, and a vasodilatory phase after volume resuscitation during prolonged and severe HH.

Several techniques for measuring CO are frequently used in humans but are unsuitable for rats (18). The transit-time ultrasound (TTU) technique, which employs a small, commercially available probe that is suitable for use in the rat aorta, was used in our study to measure the CO. The TTU technique is stable and reproducible and has low variability. It has been validated during rest and exercise (1), in chronic and acute experiments (1, 18), and by comparisons with other well-known techniques (18, 20). The probe itself does not alter dynamic blood flow measurements (18).

In our study, continuous real-time monitoring of TPR was calculated using MAP and CO. Online TPR determination could include central venous pressure (CVP) measurements. We did not use this approach, because in our experiments, CVP measurements were periodically interrupted for blood sam-

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Fig. 2. Continuous TPR curves for a constant-pressure hemorrhagic hypotension protocol plotted on the same time scale as the withdrawn and reinfused volumes for one survivor and one nonsurvivor. At time 0 min, MAP reached 40 mmHg. Horizontal bar represents the entire hemorrhage period; arrow indicates the peak hemorrhaged volume; dotted vertical line represents highest TPR value.

Fig. 3. Time to maximal loss (bleeding end points; A) and time to reinfusion of Ringer l-lactate (RL infusion starts; B) as functions of the time to maximum TPR. In both plots, solid lines represent the least-squares linear regression lines; dashed curved lines represent the 95% confidence interval for the regressions; and dotted lines represent the identity lines. Both correlation coefficients (r = 0.86 and 0.85, A and B, respectively) were statistically significant (P < 0.0001); n, no. of animals.

AJP-Heart Circ Physiol • VOL 287 • NOVEMBER 2004 • www.ajpheart.org
pling. However, because the CVP fluctuations were low and were not significantly different from the control group (17), the inclusion of CVP in TPR calculation would not affect the conclusions of this study.

By applying the TPR measurement as described here, the problem of using a discrete sampling frequency based on preestablished time points can easily be overcome, and TPR_{max} can be detected and measured as demonstrated in Fig. 1. In addition, the correlation between TPR values and other physiological responses can be evaluated. Continuous data acquisition is most useful in studies of kinetics rather than steady-state events (22, 23) and during experiments in which a “steady-state” (stable) preparation is difficult to obtain and may only be temporary. The analysis of TPR showed that these conditions may exist in our model of HH. Continuous data acquisition at a high sampling rate together with TTU aortic blood flow allowed continuous TPR recording and provided enough information to differentiate between survivors and NS in our model of HH. Therefore, we suggest that continuous measurements of TPR can be used to distinguish the survivability of rats in studies of hemorrhagic shock. The peak shed blood volume has been extensively used as a marker of the transition from the compensatory to the decompensatory phase (3, 8–10). We found that the time of TPR_{max} was strongly correlated with the preestablished time points can easily be overcome, and TPR_{max} can be detected and measured as demonstrated in Fig. 1. In addition, the correlation between TPR values and other physiological responses can be evaluated. Continuous data acquisition is most useful in studies of kinetics rather than steady-state events (22, 23) and during experiments in which a “steady-state” (stable) preparation is difficult to obtain and may only be temporary. The analysis of TPR showed that these conditions may exist in our model of HH. Continuous data acquisition at a high sampling rate together with TTU aortic blood flow allowed continuous TPR recording and provided enough information to differentiate between survivors and NS in our model of HH. Therefore, we suggest that continuous measurements of TPR can be used to distinguish the survivability of rats in studies of hemorrhagic shock. The peak shed blood volume has been extensively used as a marker of the transition from the compensatory to the decompensatory phase (3, 8–10). We found that the time of TPR_{max} was strongly correlated with the

ACKNOWLEDGMENTS

The authors are grateful to Biopac Systems technical support for consultation on data acquisition and analysis.

GRANTS

This work was supported by a grant from the US Army Medical Research and Materiel Command. L. Torres was partially supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brazil).

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