The variability of blood pressure due to small changes of hematocrit

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Submitted 24 May 2010; accepted in final form 23 June 2010

Vázquez BY, Martini J, Tsai AG, Johnson PC, Cabrales P, Intaglietta M. The variability of blood pressure due to small changes of hematocrit. Am J Physiol Heart Circ Physiol 299: H863–H867, 2010. First published July 2, 2010; doi:10.1152/ajpheart.00496.2010.—The hematocrit (Hct) of awake hamsters was lowered to 90% of baseline by isovolemic hemodilution using hamster plasma to determine the acute effect of small changes in Hct and blood viscosity on systemic hemodynamics. Mean arterial blood pressure increased, reaching a maximum of about 10% above baseline (8.6 ± 5.5 mmHg) when Hct decreased 8.4 ± 1.9% (P < 0.005). Cardiac output increased continuously with hemodilution. These conditions were reached at ~60 min after exchange transfusion and remained stationary for 1 h. Peripheral vascular resistance was approximately constant up to a decrease of Hct of about 10% and then fell continuously with lowering Hct. Vascular hindrance or vascular resistance independent of blood viscosity increased by about 20% and remained at this level up to an Hct decrease of 20%, indicating that the vasculature constricted with the lowered Hct. The results for the initial 2-h period are opposite but continuous with previous findings with small increases in Hct. In conclusion, limited acute anemic conditions increase mean arterial blood pressure during the initial period of 2 h, an effect that is quantitatively similar but opposite to the acute increase of Hct during the same period.

Hematocrit (Hct) in the healthy population varies primarily as function of age and sex. Environmental factors, genetics, nutrition, level of hydration, time in the daily cycle, and seasonal variations (9) cause an additional variability, yielding a dispersion of values that, although not well documented, may be of the order of ±10% of the average for the population grouped according to age and sex.

Hct determines blood viscosity, regulating peripheral vascular resistance (PVR) and therefore, in principle, blood pressure. Increasing the viscosity of a fluid flowing in rigid tubes increases flow resistance and tube wall shear stress. However, this does not necessarily occur in blood vessels where the vessel wall shear stress due to flowing blood modulates the production of vasodilators by mechanotransduction (15, 22). Thus, in the circulation, increasing blood viscosity can produce two opposing effects on PVR: a direct effect related to hemodynamic hindrance and an opposite effect due to changes in vessel diameter.

Changes in Hct cause hemococoncentration or hemodilution, factors that affect blood viscosity and therefore vascular resistance (VR). Our understanding of these phenomena is based on the work of Richardson and Guyton (18) and Messmer et al. (16), showing how the heart and the circulation respond to changes of Hct and therefore blood viscosity. A common denominator of these studies (12) is that the induced changes of Hct were comparatively large, similar to the Hct perturbations found in hemorrhagic shock and severe anemias and the adaptation to high altitude and polycythemia. By contrast, the normal population presents a significantly smaller variability of Hct and corresponding variability of blood viscosity (11) that does not appear to influence mean arterial blood pressure (MAP) (19). However, small acute elevations of Hct (~10%) in awake hamsters and mice performed by Martini et al. (13, 14) cause a significant lowering of MAP and elevation in cardiac output (CO) but not in endothelial nitric oxide (NO) synthase knockout mice, which do not produce NO via endothelial NO synthase. These studies and those of Salazar Vázquez et al. (20) with non-oxygen-carrying, non-NO-scavenging red blood cells (RBCs) suggested that the effect due to small changes in Hct is related to the management of NO bioavailability.

The circulatory adjustments that take place in response to a small decrease of Hct have not been systematically analyzed. In this study we characterize the changes of MAP following small changes in Hct due to isovolemic hemodilution. We compare our findings with those from previous studies with small increases in Hct (13, 14) with the objective of presenting the continuum of acute cardiovascular responses due to small changes in blood viscosity within the range of variability of Hct present in the normal population. We monitored MAP and Hct after small acute isovolumic changes in Hct 2 h after the induced change in awake animals, which was a period in which the animals were hemodynamically stable.

MATERIALS AND METHODS

Animal preparation. Investigations were performed in golden Syrian hamsters (Charles River, Boston, MA). Animal handling and care were provided following the procedures outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, Revised 1996). The study was approved by the local Animal Subjects Committee. The window chamber model is widely used for studies in the unanesthetized state, and the complete surgical technique is described in detail elsewhere (4, 7). This model was used to maintain the catheters in place in awake animals and to enable a comparison with previous results with hemococoncentration.

Briefly, hamsters were prepared for chamber implantation with an injection of pentobarbital sodium anesthesia (50 mg/kg ip). After hair removal, sutures were used to lift the dorsal skin away from the animal, and one frame of the chamber was positioned on the animal’s back. A chamber consisted of two identical titanium frames. The second frame was placed on the opposing side. Arterial and venous catheters (polyethylene-50 for hamsters and polyethylene-10 for mice) were implanted in the carotid artery and jugular vein. The
catheters were filled with a heparinized saline solution (30 IU/ml) to ensure patency at the time of the experiment. Catheters were tunneled under the skin and exteriorized at the dorsal side of the neck where they were attached to the chamber frame with tape. Experiments were performed after at least 24 h and within 48 h after catheter implantation.

Inclusion criteria. Animals were suitable for the experiments if systemic parameters were within normal limits; namely, heart rate (HR) was >340 beats/min and MAP was >80 and <125 mmHg.

Systemic parameters. MAP and HR were recorded continuously (MP 150, Biopac System, Santa Barbara, CA) except during the actual blood exchange. Hct was measured from centrifuged arterial blood samples taken in heparinized capillary tubes (Readacrit Centrifuge; Clay Adams, Division of Becton Dickinson, Parsippany, NJ).

CO measurements. CO was measured with the modified thermodilution technique (2). For this procedure we used a different group of animals not investigated for changes of MAP. CO was measured after an analysis of baseline parameters (Hct, arterial blood gases, MAP, and HR). An exchange transfusion with hamster plasma was started 5–10 min later, and CO was measured again after 60 min. Measurements were taken twice only in the same animal because of the volume addition with each CO measurement (150 μl of saline). In addition to the baseline measurement, the 60-min time point was chosen because previous experiments showed that a plateau in the changes from baseline tends to occur 60 min after the exchange. The data are reported as cardiac index (CI) or CO normalized to body weight.

Experimental setup and isovolumic hemodilution. Hemodilution to a final predetermined Hct level was accomplished by isovolumic exchange of fresh hamster plasma, obtained from blood collected from the carotid artery of donor hamsters (150–200 g) with a heparinized sterile syringe. Blood was centrifuged at 2,700 rpm for 10 min, and supernatant was withdrawn with a sterile syringe without disturbing the Buffy coat and RBCs layers. Plasma was transferred into sterile tubes and stored at room temperature and used within 2 h of the experiment. The procedures for pipetting and the transferring of materials were performed in a laminar flow hood for sterility. The volume of an exchange-transfusion step was calculated as a percentage of the blood volume, estimated as 7% of body weight. Blood samples for viscosity measurements were withdrawn from the animal with a heparinized 5-ml syringe at the end of the experiment for immediate analysis.

Previous experiments by Martini et al. (13, 14) determined that the acute increases in Hct cause cardiovascular conditions to vary and reach a steady state at about 30 min after exchange transfusion and lasted in the order of 2 h. We followed the same protocol after verifying that during hemodilution, steady cardiovascular conditions were maintained during 1 to 2 h after exchange transfusion. This period, corresponding to the maximal effects associated with the Hct change, was also used to analyze the effects of small levels of hemoconcentration. Unanesthetized animals were placed in a restraining tube. They were given 30–60 min to adjust to the tube environment before baseline measurements, and blood samples were taken (MAP, HR, and Hct) and exchange transfusion was initiated.

CO was measured at the time of the maximal expected effect (90 min after exchange transfusion) to limit the use of experimental animals, since a CO determination, at any given time point, requires one experimental subject.

At the end of the observation period (100 min), Hct was measured again and blood was withdrawn from the arterial catheter for measurements of plasma nitrate/nitrite concentrations.

A group of animals was exchange transfused with blood from a donor animal at the same Hct to determine the effect of the intervention.

VR, vascular hindrance, and rate of cardiac work. PVR was calculated as the ratio of CI and MAP. Factored out from the calculation of PVR, the effect of blood viscosity yields vascular hindrance (VH) defined as the ratio between PVR and blood viscosity μ: \[ VH = \frac{PVR}{\mu} \]

Blood viscosity as a function of Hct was estimated from the empirical relationship previously reported for hamster blood (13).

The rate of production of mechanical energy by the heart (W) was calculated from the product: \[ W = CI \times MAP \]

Statistical analysis. Results are presented as means ± SD unless otherwise noted. Data are presented as absolute values and relative to levels at baseline. A ratio of 1.0 signifies no change from baseline, whereas lower or higher ratios are indicative of changes proportionally higher or lower than baseline. A comparison of baseline values from the two different groups of animals was performed using one-way ANOVA, and post hoc analyses were performed with the Bonferroni’s multiple comparison test. Data within each group were analyzed using ANOVA for repeated measures and followed by the Bonferroni’s multiple comparison tests. Changes were considered statistically significant if \( P < 0.05 \).

RESULTS

A group of five animals was hemodiluted using fresh hamster plasma to attain a mean lowering of Hct of 0.10 to determine the period following exchange transfusion that exhibited steady hemodynamic conditions. In this group Hct was lowered by 0.097 ± 0.006 of baseline at 60 min. At 90 min, Hct was 0.084 ± 0.019 below baseline, which was not statistically different from the value at 60 min. Blood pressure increased 7.1 ± 0.8 mmHg from baseline at 60 min and 8.6 ± 5.5 mmHg at 90 min. It was assumed that steady state was reached as in the case when animals were subjected to a small hemoconcentration in the experiments of Martini et al. (13, 14). In all subsequent changes of Hct, blood pressure was recorded 90 min after the exchange transfusion. The concentration of nitrite/nitrate in blood at the end of these hemodilution experiments was 5.6 ± 1.1 μM, which was not statistically significantly different from baseline (4.4 ± 1.1 μM) (\( P = 0.08 \)).

In all cases, volume exchanges were isovolumic.

The average Hct for our experimental model in normal conditions was 0.479 ± 0.015% and MAP was 97.0 ± 7.5 mmHg. Decreasing Hct by 13.3 ± 0.16% (\( n = 15 \)) [%change = 100 (Hctfinal − Hctbaseline)/Hctbaseline] increased MAP by 6.5 ± 4.1 mmHg at 90 min after the start of hemodilution, a result that was statistically significant (\( P < 0.005 \)) relative to our normal population average. Decreasing Hct by 20.4 ± 2.6% (\( n = 5 \)) lowered MAP by 3.8 ± 6.1 mmHg, which was not statistically different from controls and the sham-treated group. Performing a 10% by volume exchange transfusion with fresh hamster blood did not significantly change either Hct (0.070 ± 0.022) or MAP (1.0 ± 2.9 mmHg, \( n = 7 \)).

Figure 1 shows a plot of how MAP and CI vary as Hct decreases from baseline and the result of calculating VR and VH.

Figure 2 compares the variability of MAP and CI for hemodilution with that found for hemoconcentration by Martini et al. (13, 14) using the same procedures.

Figure 3 compares the calculation of VH with the results from the study of Martini et al. (14) in the same animal model and the studies of Messmer et al. (16) in anesthetized dogs.

Figure 4 shows the calculation of the mechanical work performed by the heart for each level of hemodilution. Data from Martini et al. (13, 14) were used to obtain the same information for hemoconcentration.
DISCUSSION

The principal finding of this study is that the acute response to a small decrease (approximately −10%) of Hct induced by isovolumic hemodilution with homologus plasma in awake hamsters leads to an increase in blood pressure, an effect that was stable during the period of observation 60 to 90 min after the exchange. A decrease in Hct of 10% in hamsters is a change of Hct from 0.479 to 0.431. CO tends to be above baseline; however, it does not increase significantly until the decrease in Hct is >10%. Figures 1 and 2 present these effects and compares them with the effects on MAP and CO found by Martini et al. (13), showing that that there is a smooth and continuous transition from hemoconcentration to hemodilution for both MAP and CO (and CI). Observations were not extended beyond 90 min after hemodilution or 120 min after the start of the experiments because after this period the awake animals showed significant hemodynamic instability because of the extended confinement.

In these experiments VR does not fall with a small decrease of Hct, and therefore blood viscosity, but tends to remain normal up to the decrease in Hct of about 10%. These effects can be explained if we assume that the blood vessels tend to
initially constrict with decreasing Hct. This effect is evidenced by calculating VH, which factors out the contribution of blood viscosity from the calculation of VR as shown in Figs. 1 and 4, indicating that the vasculature is significantly constricted beyond a 10% reduction in Hct.

A review of the available data on hemodilution shows that the results of Messmer et al. (16) in part confirm findings on the change in VH for these small changes in Hct. This study reports all the relevant data from calculating this parameter in anesthetized dogs. Although relatively small changes in Hct were not systematically explored, data are reported that allow calculating changes of VH. This calculation shows that for 10% hemodilution, the increase in VH is only 50% of that found in the awake hamster experiments. Results from this study are presented in Fig. 3.

Previous findings showed that the systemic effects due to small increases of Hct could be directly related to the balance between NO production by shear stress and NO scavenging by RBCs. This suggests that that mechanisms for the effects found with the reduction of Hct may be related to the same cause. The reduction of Hct and blood viscosity should result in two phenomena with opposing outcomes: the reduction of shears stress and therefore NO production due to lowered blood viscosity and the reduction of hemoglobin concentration and increased plasma layer with the reduction of NO scavenging (3).

Hct changes are accommodated in part by variations in the plasma layer width, affecting vessel wall shear stress and endothelial-derived NO scavenging by RBCs.

Reports in the literature do not support a major role for NO in hemodilution; however, as previously noted, experimental studies are in general carried out with large levels of hemodilution, usually 50% isovolemic exchanges and in anesthetized animals (5, 10). The measurement of nitrite/nitrate concentration showed a tendency to increase with hemodilution; however, the effect was not significant, and how this affects NO bioavailability in the plasma layer is not well established.

Our results may explain in part the findings of Biju et al. (1) who report that essential hypertension correlates with anemia. These investigators found that anemia is more prevalent in patients with uncontrolled hypertension and that hemoglobin concentration correlates with the control of hypertension. A similar result was reported by Salazar Vázquez et al. (21) who analyzed the effects of an intensive treatment of patients with type 2 diabetes over a period of one year, finding that the control of glucose led to the decrease in blood pressure and the increase in Hct. De Simone et al. (6) found a negative correlation between Hct and systolic and diastolic blood pressure. Hypertension and type 2 diabetes are linked by endothelial dysfunction, which affects NO bioavailability (17). Therefore, our observation that in a healthy organism the lowering of Hct is associated with the increase of blood pressure and increased VH, probably mostly due to vasoconstriction, suggests that this effect may be aggravated when the endothelium has a curtailed response to the shear stress stimulus.

An analysis of the hemodynamic changes shows that the procedure of exchange transfusion of blood is associated with a small increase in CO (+5%) for the control intervention where blood was exchanged but Hct was not changed. This difference, however, could be inherent to the accuracy with which CO can be measured by thermodilution in these small animals. Cardiac work is increased significantly for a 10% increase or decrease in Hct, being a minimum at Hct that is slightly greater than normal. However, while the increase in workload with increased Hct is associated with increased oxygen delivery capacity (product of CI times Hct), workload is also increased when oxygen delivery capacity is reduced, showing the tendency for lower Hct to potentially increase cardiovascular risk.

In summary, small acute decreases of Hct (~10%) elevate blood pressure. This finding is counterpartie to the result that small Hct elevations lower blood pressure. Both phenomena are manifestations of a continuum in the relationship among acute changes in Hct, systemic VR, and responses of the circulation. The acute increase of Hct is associated with a positive balance in NO bioavailability, and the converse likely has a similar origin where the decreased Hct lowers NO scavenging by RBCs and shear stress due to the lowering of blood viscosity. The acute changes in hemodynamics attained in our study should return to baseline after the Hct transients subside in the time scale of hours. Therefore, these phenomena may be associated with exercise, meals, stress, ambient temperature, etc. It is not known whether a stable change in Hct would lead to a stable change in MAP. However, long-term Hct changes may also lead to counterintuitive results, following the same trend as acute changes. Age and Hct tend to be inversely correlated (8); therefore, aging may displace a healthy individual from the right to the left of the MAP/Hct curve, suggesting that the age-related decrease in Hct could be one of the natural components of hypertension. Furthermore, a
persistent lowering in Hct due to aging, iron-deficient diet, etc., may institute permanently small, lower NO levels that integrated in a time constitute a proinflammatory and prohypertensive factor.

GRANTS

This study was supported in part by the National Heart, Lung, and Blood Institute Bioengineering Research Partnership Grants R24-HL-064395 (to M. Intaglietta), R01-HL-062354 (to M. Intaglietta), and R01-HL-076182 (to P. C. Johnson).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES