Hemodilution mediates changes in renal hemodynamics after acute volume expansion in rats

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Franchini, Kleber G. Hemodilution mediates changes in renal hemodynamics after acute volume expansion in rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1670–R1676, 1998.—The present study examined the factors responsible for triggering renal hemodynamic adjustments during acute volume expansion. The renal hemodynamic effects of graded volume expansion with 0.9% saline (Sal; 1, 2, and 4% of body wt), 7% BSA solution (0.35, 0.70, and 1.4% body wt), or whole blood from a donor rat (WBL; 0.35, 0.70, and 1.4% body wt) were compared in rats anesthetized with pentobarbital sodium. Neural influences on the kidney were eliminated by vagus nerves, baro/chemoreceptor afferents, and renal nerves section, and renal perfusion pressure was controlled at constant level (–120 mmHg) throughout the experiments. In Sal- and BSA-expanded rats, renal blood flow (RBF) increased (Sal: 15, 40, 71% BSA 17, 49, 107%) and renal vascular resistance (RVR) decreased in parallel with the degree of volume expansion (RVR: Sal 17, 31, 44% and BSA: 15, 35, 54%). Renal hemodynamics remained unaltered after expansion with WBL. In rats expanded with Sal or BSA, correction of the fall of hematocrit restored RBF and RVR to control levels. Interference with tubuloglomerular feedback by urethral obstruction had no effect on the decrease in RVR with Sal or BSA. Inhibition of the vascular tone by intrarenal papaverine infusion also did not alter the renal hemodynamic response to volume expansion with Sal or BSA. These findings suggest that the changes in renal hemodynamics after acute expansion are likely mediated by changes in rheologic properties of the blood rather than by changes in active vascular tone.

renal blood flow; kidney; blood viscosity; blood volume; blood rheology

METHODS

Experiments were performed on adult male Wistar rats (260–330 g) obtained from animal facilities of Universidade Estadual de Campinas (Campinas, São Paulo, Brazil). The rats were fed a standard chow containing 1% NaCl. The rats were fasted overnight before the acute experiments, but water was allowed ad libitum.

Animal Preparation

Rats were anesthetized with pentobarbital sodium (30 mg/kg rat weight) intraperitoneally and placed on a temperature-controlled surgical table. The trachea was cannulated, and ventilation was controlled using a ventilator (Harvard Apparatus, South Natick, MA). Inspired oxygen fraction was varied to maintain arterial Po2 at 120 mmHg. Two cannulas were placed in the left jugular vein for intravenous infusions. The rat received an intravenous infusion of 3% bovine albumin solution in 0.9% saline at a rate of 15 ml·min−1·100 g rat wt−1 to replace surgical fluid losses. Catheters were placed in the right carotid artery and right femoral artery for measurement of arterial pressure and collection of blood sample. The carotid sinus and the vagus (at nodose ganglion level) nerves were sectioned bilaterally, and the kidney was denervated to minimize neural and hormonal reflex changes from influencing renal hemodynamics during volume expansion. The abdominal aorta was dissected, and micro-Blalock clamps were placed around the aorta above and below the left renal artery so that renal perfusion pressure could be controlled at 120 mmHg during the experiment. In some experimental protocols, a stretched PE-10 polyethylene tubing (approximate
external diameter of 100 mm) was placed in the left renal artery to allow intrarenal infusion of the vasodilator papaverine. A transonic ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the left renal artery for monitoring renal blood flow as described previously (13). This preparation was allowed to stabilize for 30 min before the beginning of the experiments. Anesthesia was supplemented as necessary throughout the experiments.

Flow Probe Calibration

Although transit-time ultrasonic flowmetry does not require particulate content or ionization of the monitored liquid, the 1RB series Transonic flow probe was calibrated for the range of hematocrit and blood flow encountered in the present study in an in situ rat common carotid artery preparation. The right common carotid artery was dissected free from the deep anterior neck muscles and catheterized with PE-50 cannulas in both downstream and upstream extremities. The flow probe was positioned around the free middle part of the vessel and kept stable with the aid of a micropositioner. To eliminate air bubbles and optimize the acoustic transmission, the region containing the vessel and the flow probe was filled with an appropriate gel (H-R Lubricating Jelly, Mohawk Medical Supply, Utica, NY). The upstream catheter was connected to a 50-ml plastic syringe mounted on a calibrated KDS 200 syringe pump (KD Scientific, Boston, MA). The common carotid artery was perfused with heparinized rabbit whole blood with four different hematocrit values (49, 44, 37, and 27%) at flow rates of 3, 6, 9, and 12 ml/min. Each infusion rate was maintained constant until the flowmeter readings were stable for at least 15 s. As indicated in Fig. 1, changing hematocrit values from 49 to 27% did not alter the blood flow measured by the 1RB series Transonic flow probe.

Monitoring

Pulsatile arterial pressure was continuously monitored from the catheters placed in the carotid and femoral artery using a COBE transducer (Arvada). The arterial pressure signal was amplified by a GP4A Steamtech amplifier (Steamtech, Milwaukee, WI). The amplifier output was connected to an analog-to-digital board and this to a computer loaded with a CODAS Data Acquisition software (AT-CODAS; DATAQ Instruments, Akron, OH) for continuous hemodynamics monitoring and recording. Pulsatile renal blood flow was also monitored continuously by the CODAS system from the signal of the renal artery flow probe connected to a T206 Transonic flowmeter. Each signal was recorded in individual channels and sampled at 100 Hz.

Preparation of Fluids and Donor Blood for Expansion

Saline 0.9% and bovine serum albumin 7% solutions were prepared fresh every day. Both sodium chloride and grade V serum bovine albumin were purchased from Sigma (St. Louis, MO). Because blood withdrawal stimulates release of a variety of vasoactive substances in the donor rat, the donor red blood cells were washed twice and resuspended in a Ringer solution containing 4% bovine serum albumin (13). The blood was centrifuged to remove the plasma. The red blood cells were washed with physiological saline solution (pH 7.4) and centrifuged again. This wash step was repeated twice, and the cells were resuspended in a Ringer 4% bovine serum albumin solution and titrated with 1 N sodium bicarbonate to correct for acid-base imbalances. Before use, the electrolytes, blood gases, and pH of the donor blood were analyzed to verify that all parameters were in the normal physiological range. In the experiments in which hematocrit was restored after volume expansion, blood was centrifuged at 3,000 revolutions/min for 5 min (Himac CTRD, Hitachi Koki) to create a red blood cell concentrate fraction with hematocrit in the range of 70–80%.

Blood Chemical Analysis and Hematocrit Determination

Samples of arterial blood (100 ml) were collected in glass capillary tubes (Ciba-Corning Diagnostics). Samples were analyzed for plasma sodium and potassium and arterial blood gases and pH by a Ciba-Corning 288 Blood Gas System (Ciba-Corning Diagnostics, Medfield, MA). Plasma sodium and potassium and arterial blood pH were measured by ion-specific electrodes. Hemoglobin concentration was measured using a spectrophotometric assay, and hematocrit was estimated from the equation \[ Hct = \frac{Hb \times 2.941}{6} \]

Experimental Protocols

Protocol 1. Comparison of the effects of volume expansion with saline, bovine serum albumin solutions, or whole blood on renal hemodynamics. Renal perfusion pressure and total renal blood flow were measured for 30 min during the control period in 18 animals. The rats then received progressive volume expansion with saline (1, 2, and 4% of body wt; n = 6), bovine serum albumin solution (0.35, 0.70, and 1.40% of body wt; n = 6), or whole blood (0.35, 0.70, and 1.40% of body wt; n = 6) in three steps of 15 min each with renal hemodynamics monitored continuously. The volumes were chosen to produce approximately the same degree of blood volume expansion with the various solutions (11, 23). Over the entire experimental period renal perfusion pressure was tightly controlled at

![Fig. 1. Calibration curves of 1RB series Transonic flow probe for the hematocrit (Hct) and blood flow ranges encountered in the present study.](image-url)
the same level as in the control period by adjusting the aortic micro-Blalock clamps. Arterial blood samples were collected at the end of each expansion step for blood gas measurements and chemical analysis. At the end of the experimental protocol, the animals were euthanized and the weight of left and right kidneys was determined.

Protocol 2. Hematocrit restoration after expansion with saline or bovine serum albumin solution. In these experiments renal hemodynamics were monitored during a 30-min control period. The animals (n = 12) were then expanded with saline (4% body wt; n = 6) or bovine serum albumin solution (1.4% body wt; n = 6) over a period of 20 min. After volume expansion, the rats then received an intravenous infusion of donor red blood cell concentrate with hematocrit of 0.80% until systemic hematocrit was restored to control over a 10- to 15-min period. Then renal blood flow was measured during an additional 20-min period. In these experiments oxygen supply to the kidney was estimated in all three steps (control, expansion, and hematocrit restoration) from renal blood flow and oxygen content values with the equation $SO_2 = \frac{CtO_2 \times RBF}{100}$, where $SO_2$ is oxyhemoglobin saturation, $RBF$ is renal blood flow, and $CtO_2$ is arterial oxygen content.

Protocol 3. Effects of blockade of renal vascular tone with papaverine on renal hemodynamic effect of volume expansion with saline or bovine serum albumin solution. In these experiments the rats (n = 22) received an intrarenal infusion of papaverine (beginning with 10 µg·min⁻¹·100 g rat wt⁻¹) that produced the maximal increase in renal blood flow without reducing renal perfusion pressure. After stabilization, renal blood flow and renal perfusion pressure were monitored for 15 min. Then the rat received an intravenous infusion of either saline (4% body wt; n = 6) or bovine serum albumin solution (1.4% body wt; n = 6) over 20 min as before, and renal hemodynamics were monitored. In different groups of rats the sequence of the experimental protocol was inverted. Saline (4% body wt; n = 5) or bovine serum albumin solution (1.4% body wt, n = 5) were infused over 20 min, and papaverine was infused after the expansion.

Protocol 4. Effect of urethral obstruction on the responses to volume expansion. The purpose of these experiments was to assess the effect of tubuloglomerular feedback on renal hemodynamic response to volume expansion with saline or bovine serum albumin solution (n = 12 animals). In the experiments the ureter was first ligated to block tubuloglomerular feedback. After a 30-min equilibration period, renal hemodynamics were measured during a control period of 15 min. The rats then received saline (4% body wt; n = 6) or bovine serum albumin solution (1.4% body wt; n = 6), and changes in renal blood flow were determined.

Statistics

Data are presented as means ± SE. Differences between mean values were tested with one-way ANOVA for repeated measure and Bonferroni’s multiple-range test. A $P < 0.05$ was considered significant.

RESULTS

Protocol 1. Comparison of the Effects of Volume Expansion With 0.9% Saline, Bovine Serum Albumin Solutions, and Whole Blood on Renal Hemodynamics

The effects obtained with graded expansion with 0.9% saline solution are summarized in Fig. 2. Renal blood flow increased progressively from control values of 7.3 to 8.4, 10.2, and 12.5 ml·min⁻¹·g kidney wt⁻¹ after progressive volume expansion with saline, whereas renal perfusion pressure was effectively clamped at 119 mmHg. Renal vascular resistance decreased from control levels of 17.3 to 14.4, 11.9, and 9.7 mmHg·ml⁻¹·min·g kidney wt⁻¹.

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Similar effects were seen when blood volume was expanded with 7% bovine serum albumin solution (Fig. 2). Renal blood flow increased from 5.7 to 6.7, 8.5, and 11.8 ml·min⁻¹·g kidney wt⁻¹. The sequence of the experimental protocol was inverted. Saline (4% body wt, n = 5) or bovine serum albumin solution (1.4% body wt, n = 5) were infused over 20 min, and papaverine was infused after the expansion.

Fig. 2. Renal hemodynamics after grading expansion with saline 0.9% solution (1, 2, and 4% of body wt), BSA solution [0.35, 0.70, and 1.4% of body wt (BWT)], and whole blood (WBL; 0.35, 0.70, and 1.4% of body wt). RPP, renal perfusion pressure; RBF, renal blood flow; RVR, renal vascular resistance. *P < 0.05 compared with control values.
Hematocrit decreased in parallel with the expansion to the same levels in saline- and albumin-expanded rats (Table 1).

In contrast with the experiments performed with saline and albumin solution, volume expansion with whole blood had no significant effect on renal blood flow or renal vascular resistance (Fig. 2).

Protocol 2. Effect of Hematocrit Restoration on Renal Hemodynamics of Expanded Rats

The results of these experiments are summarized in Fig. 3. Expansion with saline (4% body wt in a 20-min period) reduced hematocrit (from 47.3 to 36.8) and produced a 59% increase of renal blood flow (from 7.4 to 11.3 \(\text{ml/}\text{min/g kidney wt}^{-1}\)) and a 33% decrease in renal vascular resistance (from 16.4 to 10.9 \(\text{mmHg/}\text{ml}^{-1}\cdot\text{min/g kidney wt}^{-1}\)). After hematocrit was restored, renal blood flow and renal vascular resistance returned to values that were not significantly different from control (7.4 \(\text{ml/}\text{min/g kidney wt}^{-1}\) and 16.5 \(\text{mmHg/}\text{ml}^{-1}\cdot\text{min/g kidney wt}^{-1}\), respectively). Similar results were obtained in rats in which blood volume was expanded with bovine serum albumin solution. Hematocrit fell from 48.3 to 36 after blood volume expansion with 7% bovine serum albumin solution. Renal blood flow increased 49% (from 7.6 to 11.3 \(\text{ml/}\text{min/g kidney wt}^{-1}\)), and renal vascular resistance decreased by 30% (Fig. 3). After hematocrit was restored to control level, renal blood flow and renal vascular resistance returned to values not significantly different from control.

Arterial blood oxygen content decreased 22 (from 22.5 ± 0.3 to 17.5 ± 0.6 ml/dl of blood) and 25% (from 22.8 ± 0.4 to 17.1 ± 0.5 ml/dl of blood) in rats expanded with saline and serum bovine albumin solution, respectively. The estimated oxygen supply to the kidney, however, did not change significantly during hemodilution produced by the expansion (saline: 1.6 ± 0.3, 1.6 ± 0.2, and 1.5 ± 0.2; serum bovine albumin solution: 1.7 ± 0.3, 1.9 ± 0.4, 1.8 ± 0.4 ml \(\text{O}_2\) \(\cdot\text{min}^{-1}\cdot\text{g kidney wt}^{-1}\), in control, expansion, and restoration periods, respectively).

Protocol 3. Effects of Intrarenal Papaverine Infusion on Renal Hemodynamic Responses to Expansion With Cell-Free Solutions

The results of these experiments are summarized in Figs. 4 and 5. Infusion of papaverine increased basal renal blood flow by ~40% and decreased renal vascular resistance (Fig. 4). The expansion produced an additional increase of 40% (saline) and 44% (bovine serum albumin solution) of renal blood flow and a decrease of renal vascular resistance of 27 and 30%, respectively. Papaverine infusion after expansion with saline or bovine serum albumin solution (Fig. 5) produced an additional increase of 40% over ~40% observed with expansion alone. Also, these changes are not significantly different from the results seen in experiments of protocol 2 in which renal vascular tone was not inhibited by intrarenal infusion of papaverine.

Protocol 4. Effect of Urethral Occlusion on Renal Hemodynamics After Volume Expansion

The results of these experiments are presented in Table 2. Expansion of blood volume with 0.9% saline (4% body wt) or 7% bovine serum albumin solution (1.4% body wt) increased renal blood flow 41 and 48% and decreased renal vascular resistance 29 and 34%, respectively. The rise in renal blood flow and fall in...
renal vascular resistance were not significantly different from changes seen in the rats in protocols in which the ureters were not occluded.

DISCUSSION

The present study examined the factors contributing to the fall in renal vascular resistance after volume expansion. The results indicate, under conditions in which renal perfusion pressure was clamped at 120 mmHg and neural influence on the kidney was eliminated, that volume expansion with 0.9% saline or 7% bovine serum albumin solution produced similar changes in renal blood flow and renal vascular resistance, whereas volume expansion with whole blood had no significant effect on renal hemodynamics. Saline and albumin solution produced similar decreases in hematocrit, but volume expansion with whole blood had no effect on it. Moreover, we found that return of hematocrit to control completely reversed the changes in renal blood flow and renal vascular resistance produced by volume expansion with saline or bovine serum albumin solution. These results indicate that it is the fall in hematocrit that triggers the changes in renal hemodynamics.

The mechanism by which a fall in hematocrit might alter renal hemodynamics could be related to changes in vascular tone secondary to neural sympathetic suppression, release of systemic or local vasoactive substances, changes in tubuloglomerular feedback, or direct changes in rheologic properties of the blood. In this regard, acute blood volume expansion with saline and isoncotic solutions has been shown to inhibit the operation of the tubuloglomerular feedback (7, 14). The decrease in renal vascular resistance and the shift in renal blood flow autoregulation seen in expanded states has been attributed to this mechanism (1, 14, 22, 25). In the present study, the contribution of tubuloglomerular feedback inhibition for the fall in renal vascular resistance during expansion was examined after acute urethral ligation, which blocks this mechanism (7, 25). After acute urethral ligation, blood volume expansion with saline or bovine serum albumin solution still decreased renal vascular resistance to a level comparable to that seen in animals without urethral obstruction (~30%). This finding indicates that the changes in renal hemodynamics after blood volume expansion with saline or bovine serum albumin solution are not due to modulation of sensitivity of tubuloglomerular feedback.

Moreover, we also performed experiments in which renal vascular tone was inhibited with intrarenal infusion of papaverine. Papaverine increased renal blood flow by 40%. Nevertheless, blood volume expansion with saline and bovine serum albumin solution increased renal blood flow and decreased renal vascular resistance to the same extent seen in experiments with expansion alone. This suggests that the effects of hemodilution are not due to inhibition of vascular tone but may be related to changes in rheologic properties of the blood. One could argue, however, that because renal perfusion pressure was clamped to a constant level the vasodilatory effect of papaverine was only partial and that the remaining vascular tone could be responsible for the hemodynamic effects observed during expansion.
with saline or bovine serum albumin solution. Contrary to this, papaverine infused after the expansion produced similar changes in renal hemodynamics. In addition, this effect of papaverine on renal hemodynamics is close to the maximal vasodilatory effect reported for systemic administration without renal perfusion pressure control (14). Therefore it is reasonable to assume that the maximal vasodilatory effect with intrarenal infusion of papaverine and controlled renal perfusion pressure represents an extensive inhibition of renal vascular tone.

Presumably, a decrease in the hematocrit could mediate changes in renal vascular resistance throughout changes in blood viscosity. One could argue that viscosity is not the answer, because saline and albumin solution have the same effect on renal hemodynamics but should have different actions on blood viscosity. Although we did not directly assess the changes in the viscosity produced by saline or bovine serum albumin solutions, the expected differences in the changes of blood viscosity with saline or 7% bovine serum albumin solution should be minor. Plasma viscosity is more related to large proteins with molecular asymmetry, such as fibrinogen and some of the globulin fractions, and less with albumin fractions (5). Estimates of the influence of plasma albumin concentration on total blood viscosity (2) indicate that changes in plasma albumin concentration in the range of 2–7 g/dl change blood viscosity only by 10%. Moreover, blood viscosity is greatly dependent on the red blood cell concentration. This is well demonstrated in studies measuring in vivo blood viscosity in isolated maximally dilated cat hindlimb (8). The apparent viscosity measured under this condition has been shown to decrease ~40% simultaneously to hematocrit decreases similar to the ones observed in the present study (from ~45 to ~35%). Although the contribution of the viscous component to the vascular resistance is far more complex than simple dilution for a non-Newtonian fluid such as blood, this could account for most of the decrease in renal vascular resistance that paralleled hemodilution in the present study.

In addition to its effect on blood viscosity hemodilution necessarily diminishes the oxygen content per volume of blood. This could reduce the oxygen supply to the tissue, which triggers direct and indirect effects on circulation. However, in the present study it was observed that the reduction of arterial blood oxygen content during hemodilution was completely compensated by the increase in renal blood flow, which resulted in a constant oxygen supply to the kidney. Therefore, the changes observed in renal circulation during acute expansion with cell-free solutions are probably not related to changes in oxygen supply to the kidneys.

Overall our finding that changes in renal hemodynamics are dependent on rheologic properties of blood and not on changes in tone was quite unexpected, because, in many ways, changes in the hematocrit could alter vascular tone. For example, recent evidence (17) suggests that hemoglobin is responsible for the scavenging of NO in the circulation, thereby favoring vasoconstriction. In one of the first attempts to demonstrate that this mechanism plays a role in the control of systemic and regional hemodynamics, Cases et al. (4) infused in anesthetized dogs a crosslinked α-α hemoglobin, which has a higher half-life in the circulation, and observed a generalized vasoconstriction. The authors speculated that this phenomenon could be mediated by the scavenging of NO. According to this, the fall in hematocrit after acute expansion with cell-free solutions should decrease the uptake of NO and lead to vasodilation. On the other hand, decreased viscosity would decrease shear, which is the major stimulus for production of NO by the endothelium. Presently, we cannot eliminate this mechanism as responsible for the changes in renal hemodynamics seen with saline or bovine serum albumin solution. However, our studies in which renal vascular tone was blocked with papaverine before volume expansion suggest only a minor role for change in active tone mediating the rise in renal blood flow.

In conclusion, the results of the present study indicate that the fall in hematocrit contributes to the changes in renal hemodynamics after acute volume expansion with cell-free solutions. The important role of hemodilution on changes in renal blood flow is probably related to the fall in viscosity of the blood due to the non-Newtonian behavior of this fluid. Although the present study demonstrated the importance of changes on blood rheology to the adaptation of renal circulation during acute blood volume expansion under controlled conditions, the relative contribution of this mechanism and those acting through changes in renal vascular tone in intact animals remains to be elucidated.

**Perspectives**

Decreases in blood viscosity contribute fundamentally to the hemodynamic adaptation of systemic and renal circulation during acute volume expansion with cell-free solutions. From many different angles this purely physical phenomena satisfies the requisites of
an ideal physiological mechanism. It does not demand direct metabolic energy supply to be implemented. It is effective in the physiological range of systemic hematocrit, and its influence on renal vascular resistance may be easily reversed by restoration of the hematocrit to normal levels as demonstrated in the present study. Finally, it can be finely tuned to neural, hormonal, and local controllers of vascular tone, which may increase the efficiency of these adaptive mechanisms.

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