Intrarenal Hemodynamics in Cross-Circulated Hypervolemic and Isovolemic Rats

HARALD SONNENBERG, ANTHONY T. VERESS, AND JAMES W. PEARCE*

SUMMARY The microsphere technique was used to measure renal blood flow and intrarenal flow distribution in cross-circulated pairs of rats. One rat of each pair was made hypervolemic by infusion of blood (2.3% body weight), followed by intravenous reinfusion of urine to maintain intravascular expansion. The other rat of each pair, which also was urine-reinfused, was kept isovolemic throughout the experiment. As shown previously, blood infusion resulted in a large diuretic and natriuretic response in the hypervolemic partner, while a smaller but statistically significant response occurred in the isovolemic partner. Total renal blood flow did not change in the expanded rats and fell slightly but not significantly in the nonexpanded ones. Blood volume expansion was associated with a significant shift in microsphere concentration from outer to inner cortex. This shift was not, however, correlated with the magnitude of the renal response. The transferred natriuresis in isovolemic partners was not associated with any change in microsphere distribution. We conclude therefore that redistribution of blood flow to the inner cortex, although a feature of intravascular expansion, is not the primary determinant of volume natriuresis. In addition, the natriuretic activity which develops in the blood of hypervolemic rats can reduce sodium reabsorption in isovolemic rats without intrarenal hemodynamic changes.

RATS MADE hypervolemic by infusion of donor blood develop a natriuretic activity in their own blood which can be transferred to a normovolemic partner, causing diuresis and natriuresis in the latter.1 2 Possible mechanisms of this transferred effect include inhibition of active sodium transport in some part of the nephron, and indirect alteration of transport by redistribution of intrarenal blood flow. Increased flow to outer cortex3 or to inner cortex and medulla4 both have been suggested as possible mediators of “volume natriuresis,” the former by shifting filtration from juxtamedullary “salt-conserving” nephrons to superficial “salt-losing” ones, and the latter by washing out the normal medullary solute gradient. In this study we attempted to determine (1) whether the natriuresis following isotonic and iso-osmotic blood volume expansion in rats was related to changes in intrarenal

References

15. Chiu AT, Peach MJ: Inhibition of induced aldosterone biosynthesis with a specific antagonist of angiotensin II. Proc Natl Acad Sci USA 71: 341-344, 1974
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blood flow distribution and (2) whether the natriuretic factor, transferred to normovolemic rats, caused similar redistribution in the latter animals.

Methods

Male Sprague-Dawley rats (weight range = 310–395 g), anesthetized with Inactin (10 mg/100 g b.w., ip), were prepared surgically and cross-circulated as described previously. After tracheostomy, the left jugular vein and right carotid artery were cannulated with PE-90 tubing for subsequent cross-circulation. One femoral artery and vein were cannulated (PE-50) for blood sampling and pressure measurement and for intravenous (iv) infusion, respectively, and the bladder was catheterized via a suprapubic incision. The second femoral vein was cannulated for subsequent urine reinfusion.

The experimental protocol was as follows: after surgery, one of the two rats (designated the “donor”) received Heparin (1,000 IU) and a priming dose of 1 ml of Ringer’s solution containing 0.4 μCi of 3H-inulin into a femoral vein, followed by a constant iv infusion of the same solution at 0.02 ml/min.

One-half hour later, the carotid artery and jugular vein cannulas were connected to produce an arteroovenous shunt and the bladder catheter was joined to the second femoral vein cannula to return formed urine continuously to the circulation. Samples of urine then were collected for 1–2 minutes by interruption of the reinfusion line at the midpoint and end of a subsequent 40-minute control period. Arterial blood samples (0.1 ml) were taken immediately prior to urine collections. These collections were continued at 20-minute intervals throughout the experiment. Following the control period the arterovenous shunt was interrupted briefly and radioactive microspheres were injected via the carotid cannula to measure total renal blood flow and intrarenal distribution (see below). Fresh rat blood (2.3% of body weight) then was infused iv into the donor rat over 20 minutes, the expanded intravascular volume being maintained automatically by the bladder-to-vein shunt. At the same time, control collections of plasma and urine were begun in the second rat (the “recipient”), with surgical preparation and shunting procedures identical to those of the donor. One hour after the beginning of blood volume expansion of the donor rat and coinciding with the end of a 1-hour control period of the recipient, the first injection of microspheres was made into the recipient rat and was followed immediately by the second injection of microspheres into the donor rat. Cross-circulation of the volume-expanded donor and the normovolemic recipient was begun and continued for an additional hour to allow for the development of the renal response in the recipient. Briefly, the cross-circulation technique consists in placing the two rats on the two pans of a balance and connecting the carotid artery cannula of one rat to the jugular vein cannula of the other, and vice versa. Isovolemic exchange of blood between the partners is assured by adjusting clamps in the cross-circulation lines to maintain body weights constant. Lengths of arterial and venous cannulae were the same for both animals to prevent hemodynamic alterations due to the change from autologous to homologous arteriovenous shunting. At the end of the cross-circulation period, the recipient rat received a second injection of microspheres and the experiment was terminated.

Microspheres (15 ± 5 μm, 3H labeled with 85Sr (first injection) and 144Ce (second injection) were used. Approximately 2 μCi were dispersed in 0.2 ml of 4.5% bovine albumin in Ringer’s solution and injected through a cannula advanced into the left ventricle via the right carotid artery. In pilot experiments, injection in the usual dextran vehicle led to marked swelling of the muzzles and paws of the experimental rats. The reaction, which is apparently common with dextran solutions, undoubtedly is associated with hemodynamic alterations which may invalidate subsequent measurements of blood flow distribution. Suspension of microspheres in albumin solution eliminated this problem.) Location of the cannula tip was confirmed by postmortem dissection. To measure total renal blood flow the method of Arruda et al. was used: arterial blood was collected via a femoral cannula with a syringe pump. Ten seconds after the start of blood withdrawal the suspension of microspheres was injected, followed by a further 0.2 ml of vehicle for flushing purposes. This procedure was completed within 10 seconds and blood collection was continued to 1 minute, removing a total of 0.8–0.9 ml. The volume of plasma lost, therefore, was approximately balanced by the addition of iso-oncotic albumin solution. At the end of the experiment, the blood sample and right kidney were digested in equal volumes of concentrated HCl and total radioactiveities measured in a 2-channel gamma counter. Renal blood flow was calculated according to the formula: Renal blood flow (ml/min) = (total kidney counts/total blood sample counts) x rate of blood withdrawal (ml/min). The left kidney was hardened in formalin solution and a sagittal section 1–2 mm thick was cut. A wedge of tissue including the papilla was removed and divided into the following 5 components: (1) outer cortex, (2) inner cortex, (3) outer stripe of outer medulla, (4) inner stripe of outer medulla, (5) inner medulla and papilla. After weighing, the tissue strips were assayed for radioactivity. As expected, components 4 and 5 contained negligible radioactivity (<0.5% of total), while component 3 contained 5% on average. Changes in blood flow distribution in each animal were calculated from differences in radioactivity of 85Sr and 144Ce in the same kidney tissue segment, thus eliminating variability due to sectioning.

Urine volumes (V) were measured for each collection, and excretions of sodium (UNaV) and potassium (UKV) were measured, using flame photometry. Glomerular filtration rates (GFR) were calculated using radioactivity of 3H-inulin in plasma and urine, obtained by scintillation counting. Plasma protein concentration was estimated by refractometry. Statistical analysis of data included paired and unpaired t-tests, as well as linear regression and correlation.

Results

Changes in arterial blood pressure, hematocrit, and plasma protein concentration throughout the experiment are shown in Table 1. Blood volume expansion in the donor resulted in increases in hematocrit and protein concentration, but did not change the average blood pressure.
### Table 1  Average Blood Pressures, Hematocrits, and Protein Concentrations in Donor and Recipient Rats

<table>
<thead>
<tr>
<th></th>
<th>Donor (n = 12)</th>
<th>Recipient (n = 12)</th>
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<tbody>
<tr>
<td></td>
<td>Arteriovenous shunt</td>
<td>Cross-circulation Transfer</td>
</tr>
<tr>
<td></td>
<td>Control (40 min)</td>
<td>Expansion (60 min)</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>118 ± 2</td>
<td>119 ± 3</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44.3 ± 0.4</td>
<td>49.6 ± 0.3††</td>
</tr>
<tr>
<td>Protein (g/100 ml)</td>
<td>5.5 ± 0.1</td>
<td>6.1 ± 0.1†</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se.
* Statistically significant difference (P < 0.05) from corresponding control value.
†, †† Statistically significant difference (P < 0.01, P < 0.001) from both control and cross-circulation values.

### Table 2  Average Urine Volumes, Excretions of Sodium and Potassium, and Filtration Rates in Donor and Recipient Rats

<table>
<thead>
<tr>
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<th>Donor (n = 12)</th>
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<tr>
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<tr>
<td></td>
<td>Control (40 min)</td>
<td>Expansion (60 min)</td>
</tr>
<tr>
<td>V (µl/min)</td>
<td>12.2 ± 2.6</td>
<td>263 ± 32**</td>
</tr>
<tr>
<td>U&lt;sub&gt;Na&lt;/sub&gt; V (µEq/min)</td>
<td>0.45 ± 0.24</td>
<td>23.9 ± 3.1**</td>
</tr>
<tr>
<td>U&lt;sub&gt;K&lt;/sub&gt; V (µEq/min)</td>
<td>1.52 ± 0.17</td>
<td>6.97 ± 0.51**</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>1.11 ± 0.08</td>
<td>1.9 ± 0.14**</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se.
*, ** Statistically significant differences from control values within groups (P < 0.05 and P < 0.01, respectively).

During cross-circulation, red cell and protein concentration equalized in both donor and recipient, indicating the efficiency of the shared circulation. In donor, but not recipient rats, blood pressure rose during cross-circulation, suggesting that the isovolemic recipient may have supplied vasoactive hormones to the hypervolemic donor.

Average renal function in donor and recipient rats is given in Table 2. In donor rats intravascular expansion, maintained by urine reinfusion, resulted in marked diuresis, natriuresis and kaliuresis, associated with a doubling of filtered load. Cross-circulation with normovolemic recipients did not affect this volume response, while it did result in quantitatively smaller, but statistically significant increases in fluid and cation excretion in the latter group. This transferred response was not associated with alteration of glomerular filtration rate.

Renal blood and plasma flows, as well as filtration fractions in control and expansion periods (donor rats), or control and transfer periods (recipient rats) are shown in Table 3. Renal plasma flows (RPF) were calculated, using the hematocrit value of the blood sample collected just prior to microsphere injection. The corresponding GFR value was divided by RPF to obtain filtration fraction. No significant changes were found in blood or plasma flows in either group, although recipient rats showed a tendency toward decline of both variables. Filtration fraction increased markedly with volume expansion in donor rats, but showed no change in isovolemic recipients. The renal response to hypervolemia was not quantitatively related to the rise of filtration fraction; however, proportionate increases of filtration fraction and plasma protein concentration were observed in 8 of the 9 rats for which renal plasma flows could be calculated (Fig. 1). Statistical analysis of the data yielded significant correlation (r = 0.887, P < 0.01).

Average distribution of microspheres between outer and inner cortex is shown for both groups of rats in Table 4. Data are expressed as percentages of summed counts for segments 1-5 (see Methods). A significant shift of blood flow from outer to inner cortex was indicated in donor animals following intravascular volume expansion, while cross-circulation did not affect intrarenal flow distribution in recipient rats. Attempts were made to correlate blood flow distribution between outer and inner cortex,

### Table 3  Average Renal Blood and Plasma Flows, and Filtration Fractions in Donor and Recipient Rats

<table>
<thead>
<tr>
<th></th>
<th>Donor (n = 9)*</th>
<th>Recipient (n = 9)</th>
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<tbody>
<tr>
<td></td>
<td>Arteriovenous shunt</td>
<td>Cross-circulation Transfer</td>
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<tr>
<td></td>
<td>Control</td>
<td>Expansion</td>
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<table>
<thead>
<tr>
<th></th>
<th>Control (60 min)</th>
<th>Expansion (60 min)</th>
<th>Control (60 min)</th>
<th>Transfer (60 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBF (ml/min per g kidney weight)</td>
<td>7.46 ± 0.58</td>
<td>7.45 ± 1.07</td>
<td>8.07 ± 0.76</td>
<td>7.09 ± 0.76</td>
</tr>
<tr>
<td>RPF (ml/min per g kidney weight)</td>
<td>4.11 ± 0.31</td>
<td>3.74 ± 0.54</td>
<td>4.44 ± 0.44</td>
<td>3.80 ± 0.41</td>
</tr>
<tr>
<td>FF</td>
<td>0.249 ± 0.020</td>
<td>0.492 ± 0.046†</td>
<td>0.247 ± 0.032</td>
<td>0.289 ± 0.026</td>
</tr>
</tbody>
</table>

* Total renal blood flows were not obtained in 3 of the 12 pairs of rats.
† Statistically significant change (P < 0.01) from corresponding control value.
with diuresis and natriuresis, both during hypervolemia and during the transferred renal response. No statistically significant relationship could be found, however. Correlation analysis yielded the following results: (1) (a) donor rats, inner cortical distribution vs. urine flow: correlation coefficient $r = 0.101$, not significant (NS); (b) inner cortical distribution vs. sodium excretion: $r = 0.047$, NS; (2) (a) recipient rats, inner cortical distribution vs. urine flow, $r = -0.324$, NS; (b) inner cortical distribution vs. sodium excretion, $r = -0.344$, NS.

**Discussion**

Renal function in control and experimental periods of donor and recipient rats was comparable to that observed in a previous cross-circulation study using the same experimental protocol. The transfer of diuresis and natriuresis from a hypervolemic to a normovolemic partner confirms the earlier conclusion that a blood-borne factor is involved in the renal regulation of blood volume. However, neither in donors nor recipients was the renal response associated with redistribution of blood flow from outer to inner cortex, no quantitative relationship was evident between natriuretic and diuretic response and change in intrarenal hemodynamics. In addition, the transferred natriuresis in normovolemic recipient rats was not accompanied by significant change of blood flow distribution. We conclude from these data (1) that redistribution of blood flow to the inner cortex is not the primary determinant of volume natriuresis and (2) that the natriuretic activity, which develops in the blood of rats undergoing sustained intravascular expansion, reduces sodium reabsorption independent of intrarenal hemodynamic changes.

In hypervolemic rats the elevation of glomerular filtration rate without alteration of renal blood flow indicates afferent arteriolar dilation and efferent arteriolar constriction, raising net filtration pressure while keeping glomerular perfusion constant. Reflex dilation of precapillary sphincters and constriction of postcapillary sphincters is a well known consequence of increased stimulation of atrial stretch receptors by intravascular volume expansion. The present results suggest that the renal vasculature participates in this reflex. Such an interpretation is confirmed by Figure 1, in which increases in plasma protein concentration following blood infusion were positively correlated with increases in glomerular filtration fraction. Since formed urine was returned continuously to the circulation, the rise in protein concentration was due to translocation of fluid (but not colloid) into the interstitium as hydrostatic pressure in systemic capillaries increased. The good agreement between changes in protein concentration and filtration fraction in individual rats then indicates that volume expansion affected pressure in systemic and glomerular capillary beds to a similar extent.

The reflex adjustments of afferent and efferent arteriolar diameter also may explain in part the present redistribution of blood flow to the inner cortex with volume expansion. In comparison with superficial efferent vessels, those in the juxtamedullary area are either relatively thin-walled or have wider lumens, so that efferent vasoconstriction might be least effective in the inner cortex. Consequently, hypervolemia should result in decreased flow resistance primarily in the inner cortex, and in increased filtration rate primarily in the outer cortex. Saline infusion in both dog and rat was indeed associated with increased juxtamedullary blood flow and superficial filtration rate; however, as in the present experiments, no correlation was observed between the magnitude of the hemodynamic changes and the renal volume response.

Present results and earlier studies are thus compatible with the hypothesis that intravascular expansion causes a relative decrease in flow resistance predominantly in the inner cortical circulation while increasing net filtration pressure predominantly in outer cortical glomeruli. Both the increase in juxtamedullary blood flow and in superficial filtration rate may promote natriuresis. The determination of intrarenal hemodynamic changes.

**Table 4 Percent Distribution of Microspheres in the Kidneys of Donor and Recipient Rats**

<table>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Expansion</td>
</tr>
<tr>
<td>Outer cortex</td>
<td>66.9 ± 2.5</td>
<td>58.7 ± 2.6*</td>
</tr>
<tr>
<td>Inner cortex</td>
<td>32.8 ± 2.6</td>
<td>41.0 ± 2.7*</td>
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Outer cortex = segment 1; inner cortex = segments 2 + 3 (see Methods).

* Statistically significant difference ($P < 0.05$) from control period.
mining role in volume natriuresis, however, is played by additional nonhemodynamic factors, which include a transferable "natriuretic hormone" acting on tubular sodium transport.

References

Electrical Activity of Sinoatrial Node Cells of the Rabbit Surviving a Long Exposure to Cold Tyrode's Solution

KATSUHIDE NISHI, YUSHI YOSHIKAWA, FUMIO TAKENAKA, AND NORIO AKAIKE

SUMMARY The right atrium including the sinoatrial node was excised from the rabbit heart, immersed in Tyrode's solution, and kept at 0–3°C for several days. The tissue then was warmed to 36–37°C in well oxygenated Tyrode's solution. Transmembrane potentials from cells in the sinoatrial node region and the right atrium were recorded with microelectrodes. After 3 days of storage, the maximum diastolic potential of cells in the sinoatrial node region decreased, but the configuration of the action potential was identical to that of the pacemaker potential in the fresh preparation. After 5–7 days of storage, the sinoatrial node tissues in some preparations showed well preserved membrane potentials and pacemaker activity, while in others, the cells were electrically quiescent, or showed subthreshold oscillatory potentials of low amplitude. Transmembrane potentials could not be recorded from the right atrial tissue after storage for this period. Sinoatrial node action potentials with normal configuration were recorded from preparations stored for longer than 10 days. The sinoatrial cells in these preparations responded well to norepinephrine and acetylcholine, indicating that the reactivity of receptors was well preserved. The results provide electrophysiological evidence that cells in the sinoatrial node region can withstand long exposure to cold and retain their sensitivity to neuromediator action, but atrial myocardial cells cannot.

AN EARLY electrophysiological study on hearts stored at low temperature showed that 24 or 48 hours after immersion in cold Tyrode's solution, myocardial cells and cells in the conduction system generated resting and action potentials identical to those recorded prior to cooling.1 Biochemical investigations have revealed that oxygen consumption of the mammalian heart is lower in the conduction system than in the ordinary myocardium.2 3 More recent studies on hearts exposed to anoxia or subjected to coronary occlusion have shown that cells in the conduction system are more resistant to anoxia or ischemia than other myocardial cells.4 5 In addition, pacemaker cells in the rabbit sinoatrial node are the most resistant to cooling and continue to discharge at a low temperature at which all conducted action potentials and mechanical contractions have ceased.6 This suggests that electrical activity of pacemaker cells may not be drastically affected by the metabolic processes which maintain the partition of ions across the cell membrane in other myocardial cells. These observations led us to suggest that cells in the sinoatrial node also may be able to withstand a long exposure to Tyrode's solution at a low temperature, and in this way may differ from ordinary atrial myocardium.

Methods
Albino rabbits of either sex, weighing about 2 kg, were studied. The heart was removed from the animal under ether anesthesia. Samples of the right atrium with the sinoatrial node region intact were excised from the heart and immersed in 200 ml of cooled Tyrode's solution of the

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