Intrarenal Hemodynamics in Obstruction/Siegel et al.

SUMMARY To examine the role of intrarenal hemodynamics in obstructive nephropathy, we determined cortical blood flow distribution (CBFD) in rats with bilateral ureteral occlusion (BUO) and unilateral ureteral occlusion (UUO) during and after release of obstruction. Prior to release of obstruction of 24 hours’ duration, we found that outer cortical perfusion decreased by 20 ± 5% in both BUO and UUO rats. Furthermore, one hour after release of BUO, there was rapid normalization of CBFD associated with a modest return of glomerular filtration rate (GFR), an almost complete return of renal blood flow (RBF), and a marked postobstructive diuresis. In contrast, after release of UUO, we observed that outer cortical perfusion remained decreased by 21 ± 3%, both GFR and RBF remained markedly depressed, and no diuresis occurred. These data demonstrate (1) marked ischemia of the outer cortex in both BUO and UUO during obstruction, (2) a rapid return of CBFD to a normal pattern after release of BUO, but (3) persistent outer cortical ischemia following release of UUO.

Despite availability of an adequate and reproducible animal model, the mechanism responsible for the acute renal injury resulting from obstruction of the collecting system and the factors involved in the recovery process following release of the obstruction remain obscure. It has been well documented in the rat that a postobstructive diuresis follows release of bilateral ureteral occlusion but does not occur following release of unilateral ureteral occlusion (UUO) in the presence of an intact contralateral kidney. This observation offers a unique opportunity to investigate those factors that may be involved in obstructive nephropathy by studying rats that have been subjected to a similar renal injury, i.e., complete ureteral occlusion, but may have a different sequence or pattern of recovery.

Recent studies have focused attention on the role of renal hemodynamics and renal vascular resistance in obstructive nephropathy. In these studies, changes in the pattern of renal cortical perfusion have not been completely appreciated because (1) animals were studied only after release of obstruction but not during the period of obstruction, and (2) a comparison of changes in animals with unilateral occlusion and animals with bilateral occlusion has not been made using the same techniques for determination of cortical blood flow distribution (CBFD). Consequently, the present study was undertaken to determine (1) whether the pattern of cortical perfusion prior to release of obstruction was similar in rats with UUO and BUO; (2) whether alterations in renal CBFD are associated with the diuresis that follows relief of bilateral ob-
striction; and (3) whether similar alterations in renal cortical perfusion and renal blood flow would occur after release of unilateral obstruction that is not associated with a postobstructive diuresis.

Methods

Experiments were performed on male Sprague-Dawley rats (Charles River Breeding Laboratories) weighing 200-300 g. Three groups of rats were studied: group I served as controls, and a sham operation was performed; group II rats underwent complete BUO by ligation of the trigone of the bladder with a silk ligature through a small midline incision; in group III, complete UUO was achieved by placing a ligature around the left ureter near the bladder. All rats were weighed before and 24 hours after operation. Blood urea nitrogen (BUN) was measured prior to release of obstruction in all rats.

In 96 rats (28, group I; 48, group II; and 20, group III) CBFD was determined with labeled radioactive microspheres (15 ± 5 μm, 3M Co.). The microspheres were diluted with normal saline to a final concentration of 60,000 per 0.1 ml, and 1 drop of polysorbate 80 (Tween 80) was added. Immediately before injection, each sample was mixed for 1-2 minutes by an ultrasonic dismembranator (Artek Systems). In these experiments the following protocol was followed. The rats were anesthetized (pentobarbital, 30 mg/kg, ip), the left carotid artery was catheterized, and 0.1 ml of microspheres labeled with 85Sr was injected. The artery was ligated and the rats were returned to their cages. Two days later the appropriate operation for either group I, II, or III was performed under light ether anesthesia, and the rats were returned to their cages and allowed free access to food and water. Twenty-four hours later the rats were anesthetized with 5-sec-butyl-5-ethyl-2-thio-barbituric acid (Inactin) (50-100 mg/kg, ip; Promonta, Hamburg), the left carotid artery was thrombectomized, blood pressure was measured with a mercury monometer, a microhematocrit was obtained, and blood urea nitrogen (BUN) was measured prior to release of obstruction in all rats.

In 34 rats (12, group I; 14, group II; and 8, group III) glomerular filtration rate (GFR) and renal blood flow (RBF) were determined 1 hour after relief of ureteral occlusion of 24 hours' duration. These rats were anesthetized (Inactin, 50-100 mg/kg, ip), a tracheostomy was performed, and polyethylene catheters were secured in the external jugular vein, left carotid artery, and for the collection of urine as described. After replacement of surgical fluid lost with isotonic saline (1% body weight), the blood pressure was measured with a mercury manometer, a priming dose of 10 μCi of 3H-methoxy-inulin (New England Nuclear) was given and followed by a sustaining infusion of 10 μCi/hour in a volume of 1.2 ml. After a 60-minute equilibration period, inulin clearance was determined by the average of three 10-minute urine collections. Blood samples were obtained from the tail at the midpoint of each urine collection, and at the end of the third clearance period a sample was obtained from the left renal vein (groups I and II) or both renal veins (group III). The concentration of 3H-methoxy-inulin was determined with a Tri-Carb liquid scintillation counter, and the inulin clearance and RBF were calculated with standard formulas as previously described.

All values are means ± sem, and Student's t-test was used to determine the significance of differences.

Results

The total number of rats in each group and the characteristics of the groups after 24 hours of complete ureteral occlusion are shown in Table 1. The final body weight (which was the weight 24 hours after the appropriate operation for group I, II, or III) and the change in body weight (which was the final body weight minus the weight at the time of initial surgery) were similar for all three groups (P = NS). All of the rats lost weight after either sham operation or ureteral occlusion despite being allowed free access to food and water. Also, the hematocrit and blood pressure were similar in the three groups (P = NS). As expected, the BUN concentration was signifi-

cated by proportional flow 141Ce/proportional flow 85Sr. Since no change in CBFD would give a ratio of 1.00, the percentage change in proportional flow would be given by:

\[
\left( \frac{\text{proportional flow}^{141}\text{Ce}}{\text{proportional flow}^{85}\text{Sr}} - 1 \right) \times 100.
\]

Because the proportional flow to the midcortex (zone B) was essentially unchanged (P not significant [NS]) in each group under all experimental conditions, the values for CBFD are expressed as the percentage change in proportional flow of outer cortex (zone A) and deep cortex (zone C). Previous studies have demonstrated that this method is reproducible, that the microspheres are removed in a single circulation, and that the initial preparation and injection do not alter intrarenal hemodynamics.

In group I rats a bladder catheter (PE-50) was used to collect urine; in group II the obstruction was relieved by catheterizing the dilated ureters (PE-50); and in group III the dilated left ureter was catheterized and a bladder catheter was used to collect urine from the right (contralateral) kidney.

In 34 rats (12, group I; 14, group II; and 8, group III) glomerular filtration rate (GFR) and renal blood flow (RBF) were determined 1 hour after relief of ureteral occlusion of 24 hours' duration. These rats were anesthetized (Inactin, 50-100 mg/kg, ip), a tracheostomy was performed, and polyethylene catheters were secured in the external jugular vein, left carotid artery, and for the collection of urine as described. After replacement of surgical fluid lost with isotonic saline (1% body weight), the blood pressure was measured with a mercury manometer, a priming dose of 10 μCi of 3H-methoxy-inulin (New England Nuclear) was given and followed by a sustaining infusion of 10 μCi/hour in a volume of 1.2 ml. After a 60-minute equilibration period, inulin clearance was determined by the average of three 10-minute urine collections. Blood samples were obtained from the tail at the midpoint of each urine collection, and at the end of the third clearance period a sample was obtained from the left renal vein (groups I and II) or both renal veins (group III). The concentration of 3H-methoxy-inulin was determined with a Tri-Carb liquid scintillation counter, and the inulin clearance and RBF were calculated with standard formulas as previously described. All values are means ± sem, and Student's t-test was used to determine the significance of differences.

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Obstruction (upper panel) and 1 hour after release of obstruction (lower panel) in sham-operated (group I), bilaterally obstructed (group II), and unilaterally obstructed (group III) rats. In group III rats the left kidney was the obstructed (experimental) kidney. All values are mean ± SEM.

**TABLE 1** Characteristics of Groups after 24 Hours of Complete Ureteral Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Sham-operated</th>
<th>Bilateral obstruction</th>
<th>Unilateral obstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body wt (g)</td>
<td>258 ± 8.9</td>
<td>255 ± 8.7</td>
<td>242 ± 8.2</td>
</tr>
<tr>
<td>Δ body wt (g)</td>
<td>-5.6 ± 0.8</td>
<td>-7.0 ± 1.2</td>
<td>-8.6 ± 1.4</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>46 ± 0.6</td>
<td>45 ± 0.8</td>
<td>47 ± 0.9</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>107 ± 3</td>
<td>110 ± 2</td>
<td>107 ± 1</td>
</tr>
<tr>
<td>BUN (mg/100 ml)</td>
<td>20 ± 5</td>
<td>88 ± 5*</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Total number of rats</td>
<td>40</td>
<td>62</td>
<td>28</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. Final body weight = weight 24 hours after operation. Δ body weight = body weight 24 hours after operation minus body weight prior to operation.

*P < 0.01 compared to sham-operated and unilateral obstructed rats.

The percent change in proportional flow 1 hour after release of obstruction is shown in the bottom panel of Figure 1. In group II rats the CBFD had reverted to a normal pattern, and the changes in fractional outer and deep cortical perfusion were not significantly different from control (group I) (P = NS). However, the fractional outer cortical perfusion in the left kidney of group III rats remained significantly decreased, -21 ± 3% (P < 0.01), and proportional deep cortical perfusion remained increased, +13 ± 2% (P < 0.01). The changes in the contralateral kidney in the group III rats were not significant (P = NS). Urine flow rates in these rats averaged 1.76 ± 0.21 μl/min per 100 g of body weight per kidney in group I, 14.61 ± 2.82 in group II, and 0.96 ± 0.28 in the experimental (left) kidney and 1.94 ± 0.31 in the contralateral kidney of group III.

A comparison of the changes in CBFD prior to release of ureteral occlusion to those alterations in CBFD 1 hour after release of obstruction is particularly important (Fig. 1, upper panel compared to lower panel). In group I the changes were similar at both times and not different from zero. However, in group II there was a marked change in CBFD during the 60 minutes after release of obstruction. During this time interval in group II rats the proportional flow to the outer cortex increased and the proportion of flow to the deep cortex decreased so that the pattern of CBFD resembled that in the sham-operated rats. These represent distinct and important changes in the CBFD pattern as compared to CBFD prior to release of obstruction. In the group III rats, however, the pattern of CBFD prior to and following release of obstruction remained unchanged and was characterized by decreased fractional outer cortical perfusion and increased deep cortical perfusion. During the 1 hour after release of the left ureteral obstruction, there was no change in CBFD in the group III rats. Thus, in group II rats there were distinct and significant changes in CBFD following the release of bilateral ureteral occlusion, whereas in group III no changes in CBFD occurred and the pattern of CBFD remained significantly different from control (group I). These changes in the pattern of CBFD following release of BUO (group II) were associated with a massive increase in urine volume, a postobstructive diuresis, which was not observed for group I or III rats.

GFR and RBF as determined by inulin clearance and
Discussion

Over the past several years it has been commonly observed in the rat that a postobstructive diuresis follows release of BUO of 24 hours' duration,1-10 but does not occur after release of UUO in the presence of an intact contralateral kidney.6-8 Despite this difference in the magnitude of losses, similar defects in tubular function, i.e., increased fractional sodium excretion (CNa/GFR) and decreased free water reabsorption (TCH2O/GFR) occur in the injured kidney of both UUO and BUO rats.5-7,9 Consequently, it may be possible that similar mechanisms are responsible for the renal injury during ureteral occlusion in both UUO and BUO but that differences in the rate of change or the magnitude of changes following release of obstruction are responsible for the observed differences during recovery in UUO and BUO rats. Data from the present studies would support such a hypothesis with regard to the role of CBFD in obstructive nephropathy.

During the 24 hours of obstruction but prior to release (upper panel of Fig. 1) there was a marked and significant decrease in fractional outer and a slight increase in deep cortical blood flow in the experimental kidneys of both BUO (group II) and UUO (group III) rats. A similar distribution of fractional blood flow to superficial and juxtamedullary nephrons has been noted during acute (less than 1 hour) UUO12-14 but has not been previously appreciated prior to release of obstruction of 24 hours' duration. Although in the present studies total RBF was not measured during the period of complete ureteral occlusion, studies by Vaughan et al.16 and, more recently, by Yarger and Griffith9 have demonstrated that in dogs with UAO at the end of 24 hours of obstruction RBF had decreased to a value between 25% and 50% of the preobstruction values. In addition, preliminary data from our laboratory suggest that total RBF and glomerular perfusion in the superficial cortex are both markedly reduced after 24 hours of ureteral occlusion in the rat.16 Thus, it would appear that similar mechanisms are operative during the period of obstruction in the experimental kidney of both UO and BUO animals, and after 24 hours of obstruction there is marked ischemia of the outer cortex in kidneys with complete ureteral occlusion.

By use of micropuncture techniques to determine changes in nephron function in the rat, Yarger et al.,5 McDougall and Wright,9 and Jaenike1 all have shown decreased superficial nephron GFR and defects in both proximal and distal tubular function following release of obstruction. In addition, Yarger and colleagues5,9 and Wilson17 have demonstrated marked heterogeneity of nephron function in the postobstructive kidney. More recently, Sonnenberg and Wilson18 have proposed that the net addition of sodium to the medullary collecting duct fluid is a direct effect of obstruction, since this effect was observed after relief of BUO and UUO but not with urine reinfusion alone. These defects in nephron, tubular, and collecting duct function could have been induced by an ischemic injury of the outer cortex during the period of obstruction but were not shown, in any of these studies, to have resulted from such an injury.

In the present studies, 1 hour after release of obstruction in the BUO (group II) rats the pattern of cortical perfusion (lower panel of Fig. 1) was similar to that in the sham-operated rats. It is important to point out that, while the CBFD at this time was not significantly different from control values, this pattern represented a marked change from the CBFD prior to release of obstruction. Thus, in the BUO (group II) rats there was a reperfusion of the previously ischemic outer cortex during the 60 minutes after relief of obstruction. This reperfusion of the outer cortex was associated with (1) a marked diuresis, (2) a modest return of GFR, and (3) an almost complete improvement in total RBF. Interestingly, Jaenike1 has previously reported that total RBF and cortical distribution of

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**Table 2 Renal Function and Blood Flow 60 Minutes after Release of Obstruction**

<table>
<thead>
<tr>
<th></th>
<th>Sham-operated</th>
<th>Bilateral obstruction</th>
<th>Obstructed</th>
<th>Contralateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (μl/min per 100 g body wt)</td>
<td>1.83 ± 0.24</td>
<td>12.5 ± 1.8*</td>
<td>0.75 ± 0.18*</td>
<td>2.26 ± 0.65</td>
</tr>
<tr>
<td>GFR (μl/min per 100 g body wt)</td>
<td>587.5 ± 27.3</td>
<td>145.3 ± 26.4*</td>
<td>80.9 ± 12.4*</td>
<td>606.9 ± 33.7</td>
</tr>
<tr>
<td>RBF (μl/min per 100 g body wt)</td>
<td>3056.0 ± 235.9</td>
<td>2384.3 ± 116.6†</td>
<td>1229.4 ± 49.1†</td>
<td>3231.9 ± 222.9</td>
</tr>
<tr>
<td>U/P inulin</td>
<td>368.2 ± 60.9</td>
<td>12.6 ± 2.6*</td>
<td>14.5 ± 2.8*</td>
<td>360.6 ± 60.7</td>
</tr>
<tr>
<td>No. of rats</td>
<td>12</td>
<td>14</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

All values are mean ± SEM for one kidney.

V = urine flow rate; GFR = glomerular filtration rate; U/P inulin = ratio of urine to plasma inulin.

* P < 0.01 compared to sham-operated group.

† P < 0.05 compared to sham-operated group.
flow are normal 1 hour after release of BUO in female Wistar rats, but he had failed to appreciate the significance of these changes relative to the period of obstruction because he did not study renal hemodynamics during obstruction. Thus, in the present studies, the recovery phase in rats with BUO was characterized by a preferential reperfusion of previously ischemic outer cortical nephrons which resulted in a marked diuresis and progressive increase in GFR. It is possible that a disproportionate increase in the filtered load of solutes and water in the outer cortical, short-looped nephrons plays an important role in the postobstructive diuresis. The polyuria that characterizes the recovery phase in these rats with BUO may be the result of an inability of the damaged tubules to reabsorb a proportional amount of filtrate, indicating glomerular tubular imbalance as a result of a restoration of perfusion and glomerular filtration before return of tubular and collecting duct function. Similar changes in CBFD have been observed in our laboratory during the diuretic phase of the recovery process in rats with ischemic or dichromate-induced acute renal failure. Therefore, the pattern of recovery observed in the BUO rats may not be specific for obstructive nephropathy but may represent a pathophysiological mechanism that occurs during the recovery phase in a number of conditions that are characterized by (1) a decrease in outer cortical perfusion during the acute injury reaction, and (2) a polyuria during the early recovery period.

Of particular importance and in sharp contrast are the findings 1 hour after release of UUO (group III). In these rats the pattern of CBFD in the experimental (left) kidney 1 hour after release of obstruction was unchanged from the CBFD pattern during obstruction (Fig. 1). Both prior to and 1 hour after release of UUO there was a significant decrease in outer cortical perfusion of the left kidney. In these rats no diuresis occurred and both GFR and RBF remained markedly depressed. Thus, in these rats, the cortical hyperperfusion that was present during the 24 hours of obstruction persisted after relief of obstruction, and the recovery process was blunted. These observations are similar to the findings of Harris and Yarger, who noted that, after relief of UUO in either rats or dogs, there was severe renal vasoconstriction and multiple scattered areas of nonfilling of glomerular and peritubular capillaries in the outer cortex. Interestingly, Sonnenberg and Wilson found that attempts to determine collecting duct function in animals with UUO were unsuccessful because of extremely low urine flow rates from the experimental kidney. Thus, although CBFD during the period of obstruction in the experimental kidney of animals with UUO is similar to that of animals with BUO and is characterized by a decrease in fractional outer cortical perfusion following release of obstruction, reperfusion of the ischemic outer cortical nephrons does not occur in animals with UUO as compared to animals with BUO, the recovery process is delayed, and a postobstructive diuresis does not occur.

Since the BUN was significantly greater in the BUO rats than in the sham-operated or UUO groups, it is possible that urea may have been responsible for the difference in CBFD and urine volume following release of obstruction. Yarger et al., Honrath, and Harris and Yarger have shown that the intravenous infusion of urea does not produce a postobstructive diuresis in anesthetized UUO animals. Although Harris and Yarger and Wilson and Honrath have proposed that urea infusion may partially reverse the renal vasoconstriction that follows ureteral obstruction, these effects could be demonstrated only in animals which were (1) awake, (2) volume-loaded, or (3) reinfused with urine. Thus, in our present series of experiments in which the rats were all anesthetized and neither volume-loaded nor reinfused with urine, it seems unlikely that the level of urea alone could have been responsible for the difference in CBFD seen in BUO and UUO rats.

Although the mechanisms responsible for a postobstructive diuresis remain undetermined, several observations appear well established. Ureteral occlusion of 24 hours' duration results in reduced filtration and decreased fractional reabsorption of fluid and sodium. The studies of Harris and Yarger and recent observations by Wilson and Honrath from cross-circulation experiments suggest that changes in blood composition may be responsible for the tubular defects. In addition, Sonnenberg and Wilson have shown that ureteral occlusion produces an intrinsic defect in collecting duct function that results in a rise in sodium and water to tubular fluid. The results of our present experiments establish important and previously unappreciated differences between the pattern of renal cortical perfusion following release of BUO and UUO. In addition, these studies emphasize the importance of preferential reperfusion of the renal cortex in order to reestablish filtration and, thereby, allow the tubular and collecting duct defects to become manifest as a postobstructive diuresis.

Several observations from the present studies are of particular note: (1) during ureteral obstruction there is a marked decrease in proportional blood flow to the outer cortex in both UUO and BUO rats, (2) after relief of BUO, there is a rapid return of CBFD to a normal pattern and a decrease in renal vascular resistance which is associated with a postobstructive diuresis, and (3) similar changes in intrarenal hemodynamics do not occur after release of unilateral obstruction, suggesting that the changes observed after release of bilateral obstruction may be causally related to the recovery process which is occurring in that animal. Currently, several investigators are searching for a factor or substance which is either retained in the blood during BUO or excreted in the urine during UUO and which may be responsible for the pathogenesis of a postobstructive diuresis. The changes in CBFD described in the present investigations represent a pathophysiological mechanism through which such factors may be operative and indicate that the effect on renal vascular resistance and CBFD should be determined for any substance or factor which is thought to be responsible for a postobstructive diuresis.

Acknowledgments

We thank Sonia Gunstream for expert technical assistance and Esther Nichols for invaluable secretarial help.
Directional Coronary Collateral Growth with Chronic Circumflex Occlusion in the Dog

KONRAD W. SCHEEL, REGINALD J. RODRIGUEZ, AND LESLIE A. INGRAM

SUMMARY The object of this study was to determine whether coronary collateral resistances were dependent on the direction of perfusion and to investigate whether a pattern of collateral growth with gradual circumflex occlusion could be discerned. In 12 dogs an Ameroid occluder was placed on the circumflex for 1 month, and in six dogs for 3 months; 12 dogs served as controls. The circumflex, left anterior descending, and right coronary arteries were separately but simultaneously perfused in an isolated heart preparation. The results showed that collateral flows from the right to the left coronaries in control dogs were 3.5-fold larger than when these collateral beds were perfused in the opposite direction. This difference in the 1- and 3-month Ameroid groups was approximately 20-fold. Relative to the control group, the collateral resistances from right to left coronary vessels were an average of 10-fold less in the 1- and 3-month groups, but there was no significant difference in resistance in the collaterals perfused from the left to the right. The results strongly suggest that collateral proliferation occurs in response to hypoxia rather than to a pressure gradient, and that collateral development is toward the hypoxic area.

From the Department of Physiology and Biophysics, University of Tennessee Center for the Health Sciences, and the Department of Electrical Engineering, Christian Brothers College, Memphis, Tennessee. Supported by Grants HL-15623 and HL-09495 of the National Institutes of Health, U.S. Public Health Service, and the Tennessee Heart Association.

Address for reprints: Dr. Konrad W. Scheel, University of Tennessee, Center for the Health Sciences, Department of Physiology, 951 Court Avenue, Room 456D, Memphis, Tennessee 38163.

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References

67 pressure gradients, and radial and tangential stresses on the vessel, the precise mechanism continues to elude investigators. Previous studies\textsuperscript{11}\textsuperscript{12} have shown that collateral or retrograde flow to a coronary vessel that had been gradually occluded increased considerably with time. It has also been realized that the source for collateral flow consists of the sum of the contributions from the other major coronary vessels. The purpose of this study was to establish a method that allows a separation of the collateral beds between the major coronary vessels and to determine whether a pattern for vascular proliferation could be discerned. In an isolated heart preparation the three major coronary arteries were perfused separately but simultaneously under conditions of maximum vasodilation. The collateral resistances were determined from measurements of retrograde flows made on two vessels simultaneously.
Renal cortical blood flow distribution in obstructive nephropathy in rats.

N J Siegel, R A Feldman, B Lytton, J P Hayslett and M Kashgarian

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