Collateral Circulation after Renal Artery Occlusion in the Rat

M. Barry Siegel and Norman G. Levinsky

SUMMARY We used angiographic and microsphere methods to evaluate the anatomic and functional features of renal collateral circulation in the rat. By the microsphere method, renal parenchymal blood flow was less than 1% of control 1 hour after occlusion of the main renal artery; 2.8% of control 1-2 weeks after arterial occlusion; and 1% of control 4-9 weeks after occlusion. Radiographic observations during chronic occlusion revealed numerous collateral vessels to the kidney. These vessels readily filled with angiographic contrast medium but the intrarenal circulation did not visualise. We conclude that collateral circulation to renal parenchyma is negligible after acute or chronic occlusion of the main renal artery in the rat. The rich anatomic plexus of collateral vessels has no functional significance and is unable to preserve viability of the parenchyma.

MANY ANATOMICAL and radiographic studies demonstrate that collateral vessels are present after occlusion of the renal artery in the rat and the dog. However, there are few measurements of the actual rate of blood flow through these collateral channels. To evaluate the functional significance of collateral circulation in the rat, we have visualized collateral vessels angiographically and quantitated nutrient flow to the renal parenchyma with microspheres. Although impressive collateral vessels develop during arterial occlusion, parenchymal blood flow is negligible.

Methods

MEASUREMENT OF RENAL BLOOD FLOW WITH MICROSPHERES

For chronic studies, male Sprague-Dawley rats, weighing 210-400 g and allowed free access to food and water, were anesthetized with methohexitol (100 mg/kg). A left flank incision was made and the renal artery was carefully separated from the renal vein near its origin from the abdominal aorta. Care was taken not to disrupt perirenal structures. The renal artery was tied at two separate locations with 4-0 silk. The proximal tie was as close to the aorta as possible and was proximal to any identifiable branches of the renal artery. Any rats in which the entire kidney did not blanch uniformly were discarded. Five to 60 days later, these rats were anesthetized with Inactin (ethyl-(l-methyl-propyl)-malonylthiourea) (100 mg/kg, ip). A tracheostomy tube (PE 240) was inserted and the right jugular vein was cannulated with PE 50 tubing for infusion of heparinized saline at 0.028 ml/min (6 units of aqueous heparin/ml). A second PE 50 cannula was placed into the right femoral artery and connected to a pressure transducer and recorder. A third PE 50 cannula was placed into the left axillary artery and advanced to the junction of this artery and the aorta. Rats were maintained at normal body temperature throughout the experiment by use of thermostatically controlled heating coil and esophageal sensing probe.

One-half hour after the above surgery had been completed, a timed 60-second free-flow collection of blood was begun from the right femoral artery cannula under oil into a preweighed plastic test tube. The total volume of blood collected was 1 ml or less. Five seconds after this timed collection began, 0.25-0.3 ml of 85Sr-labeled microspheres (15 ± 5 μm; 100,000-440,000 microspheres/ml, 10% dextran; specific activity, 9.88 mCi/g) were injected rapidly via the left axillary artery cannula; this was followed quickly by 0.5 ml saline to flush the line. The microspheres were sonicated with an ultrasonic probe for 1 minute just prior to injection. The injection of microspheres and saline took no longer than 10 seconds. After the femoral artery blood collection had been completed, the appearance of the left kidney and adequacy of the renal artery occlusion were noted. Both kidneys and a section of left lung were removed.

Each kidney was handled separately, the capsule stripped and ureters as well as hilar vessels removed. A midsagittal section of the kidney was made and the entire papilla removed. The remaining renal tissue was divided into two or three portions, each weighing less than 1 g. The samples then were weighed and placed into individual plastic counting tubes, each containing 1 ml of water. The papilla was weighed and counted separately to assess any papillary blood supply. Each sample then was counted for 10 minutes in a three-channel gamma spectrometer.

For acute studies, the protocol was essentially the same as that described for the chronic occlusion experiments. After the initial surgery, the rat was placed on its right side and a left flank incision made. With minimal dissection the left renal artery was carefully dissected away from the left renal vein at its origin from the aorta. Ten to 15 minutes later, the left renal artery was occluded with a modified Blalock clamp. Thirty minutes after occlusion, 0.05-0.08...
ml of 5% Lissamine Green was injected iv and the occluded kidney observed with a stereomicroscope. The animal was discarded if any Lissamine Green appeared on the surface of the occluded kidney. Sixty minutes after occlusion, a femoral artery collection was begun and subsequently microspheres were injected as described for the chronic occlusion experiments. The rest of the protocol was identical to that for the chronic occlusion experiments.

The dose of microspheres used for all experiments was chosen to allow detection of blood flow rates in the ischemic kidney as low as 0.5% of that to the contralateral, control kidney. Relative flow in the two kidneys of each rat was calculated directly from the total radioactivity of each kidney. In addition, absolute blood flow to each kidney was estimated using the femoral artery blood collection as a reference:

\[ \text{RBF} = \frac{M_a F_{fa}}{M_k} \]

where \( \text{RBF} = \text{renal blood flow, ml/min} \)

\( M_a = \text{cpm in femoral artery blood sample} \)

\( M_k = \text{cpm in whole kidney} \)

\( F_{fa} = \text{femoral artery blood flow, ml/min} \)

All results are expressed as mean ± SEM. Statistical significance was determined by a paired t-test; each ischemic kidney was compared to its contralateral control.

### AORTIC ANGIOGRAMS AFTER CHRONIC RENAL ARTERY OCCLUSION

In eight rats, the renal artery was occluded 7-62 days before use, exactly as described for the chronic microsphere experiments. On the day of study, rats were anesthetized and placed on a heated table. A tracheotomy was performed, and the rat was infused with heparinized saline iv. A midline abdominal incision was then made and loose 4-0 silk ties put around the proximal portions of both the superior mesenteric and celiac arteries. The bowel then was mobilized as much as possible, moved lateral to the left kidney, and wrapped in gauze moistened with warm normal saline. The distal aorta and inferior vena cava were dissected and loose ties were put around these vessels. A small bulldog clamp then was placed temporarily on the aorta just proximal to its bifurcation and an incision was made distally. A saline-filled PE 160 cannula was placed into the aorta and advanced proximally to a position 1-1 ½ cm below the renal arteries. Two thousand units of aqueous heparin were then injected iv, the celiac and superior mesenteric artery ties completed, and a sternum-splitting incision was made. Quickly, 4-0 silk ties were placed around the midthoracic aorta and thoracic inferior vena cava. A large incision was made in the distal abdominal inferior vena cava and 15-20 ml of fresh Krebs-Ringer bicarbonate solution (pH 7.4) infused into the aortic cannula until moderately clear fluid was seen flowing from the vena caval incision. At this point, an infusion of 20 ml of well mixed barium into the aortic cannula was begun and, simultaneously, rapid sequence magnified x-rays were begun at 3 films/second. Films were made for 5-8 seconds and the experiment terminated. Films were developed using standard techniques and examined for the presence of renal collateral vessels.

### RESULTS

#### DEMONSTRATION OF COLLATERAL VESSELS

**Chronic Left Renal Artery Occlusion**

In five rats, aortic angiograms were obtained 7-11 days after total occlusion of the left renal artery. The experiment will be referred to as early chronic occlusion. In the remaining three rats angiography was done 38-62 days after left renal artery occlusion. The experiments will be referred to as late chronic occlusion. An x-ray of the abdomen was taken with the rat lying supine prior to barium injection. This revealed a marked decrease in the size of the ischemic kidney and intrarenal calcification in all the rats with late chronic occlusion. In addition, the contralateral, control kidney was considerably larger than kidneys of age-matched controls. In the rats with early chronic occlusion there was no intrarenal calcification and the ischemic kidney appeared only slightly smaller than the contralateral kidney. Immediately after aortic injection of barium, prominent collateral vessels were noted in and around the occluded kidney in all rats of both groups (Figs. 1-3). The major branches of the main renal artery regularly filled by retrograde flow distal to the ligatures (Figs. 2 and 3). Collateral vessels were seen leading to these renal arterial branches as well as to the capsular vessels. They appeared to originate primarily from the ileolumbar, inferior phrenic, ureteral and lumbar arteries. Occasionally, testicular or adrenal vessels were seen to feed collaterals to a renal artery branch. The collateral vessels appeared equally prominent in the early and late chronic occlusion studies. In no rat were intrarenal vessels seen in the ischemic kidneys beyond the primary branches of the renal artery nor were nephrogram notes. In the control kidneys, the usual intrarenal arterial arborization was noted (Figs. 1-3).

#### RENAL BLOOD FLOW MEASUREMENTS (TABLE 1)

**Acute Occlusion**

After an hour of ischemia, the left kidney was hemorrhagic and swollen. Microscopically, all tubules appeared collapsed. The ischemic kidneys weighed slightly more than their controls. After arterial occlusion blood flow to the left kidney was negligible, both in absolute terms (0.03 ml/min) and in comparison to the contralateral control (0.34%). There was no measurable flow to the papilla of the left kidney; radioactivity in the papilla was not above background in any experiment.

**Chronic Arterial Occlusion**

Four experiments were made after 5-12 days of occlusion (early chronic occlusion). These kidneys were yellow-brown in appearance and weighed about two-thirds as much as the controls. There were occasional, small, pale pink areas on the anterior or posterior surface of the kidney, but no slicing was seen that these areas did not extend into the depth of the cortex. The ischemic kidney was hard, and the cut surface was "gritty." Seven rats were studied 28-60 days after arterial ligation (late chronic
Angiogram taken 7 days after total occlusion of left renal artery. Note well developed plexus of periureteral vessels to left kidney. Right intrarenal vasculature is well filled.

Discussion

In agreement with results of many other studies\(^1\)-\(^6\) which demonstrate collateral circulation after the renal artery is totally or partially constricted, our angiographic observations demonstrate numerous collateral vessels 1–10 weeks after complete arterial occlusion. We did not obtain radiological data immediately after arterial occlusion, but Donahoe et al.\(^1\) have demonstrated preexisting collaterals by angiography within an hour after division of the renal artery in the rat. These investigators found that new channels are progressively added to preexisting vessels over the period of 8–97 days after arterial occlusion. We were not impressed by any obvious increase in collateral vasculature over the 6–62 day interval we studied. However, such observations are semiquantitative at best.

Despite the striking complex of collateral vessels visualized angiographically in our chronic experiments, renal blood flow was minimal in all rats. There was no evidence of a significant progressive increase in blood flow with time. Indeed, blood flow tended to be less in rats studied 4–9 weeks after occlusion than in those evaluated in the first 2 weeks. The angiographic medium filled collateral vessels within the first 1–2 seconds after injection. It is evident that these arteries are large enough to accept rapid blood flow. While care was taken not to inject the contrast medium with undue force, perfusion pressure was not monitored during injection. Therefore, high aortic press-

After complete arterial occlusion, the cortices of these kidneys appeared to be replaced by hard, white, calcified nodules. The ischemic kidneys were greatly reduced in weight.

In the early chronic occlusion experiments, renal blood flow to the ischemic kidney was less than 3% of the blood flow to the contralateral control kidney. Absolute blood flow to the ischemic kidneys was only 0.29 ml/min. In the late chronic occlusion experiments, blood flow to the ischemic kidneys was about 1% of control. Absolute renal blood flow to the ischemic kidneys was only 0.12 ml/min. There was no detectable flow to the papilla; radioactivity was at background in all rats studied.

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sures could have developed and accounted for an overestimate of flow through collateral vessels at more normal arterial pressures. In any case, the renal parenchyma receives no significant nutrient flow through collateral channels. The microspheres used to determine blood flow are 15 microns in diameter and obviously can pass easily through collaterals of the size visualized angiographically. Blood flow to the renal parenchyma calculated by this method did not exceed 3% of normal and was 1% of normal or less in most rats. Flow through collaterals, therefore, either is at this minimal level and/or passes through channels which do not connect with the intrarenal microvasculature. As noted in Results, angiograms never revealed evidence that intrarenal vessels filled during angiography nor were nephrograms ever noted. The atrophy and calcification of the chronically ischemic kidneys noted by gross examination also are consistent with the absence of significant nutrient blood flow.

A number of authors have attributed differences in the biochemical effects on renal parenchyma of various techniques for inducing ischemia to the presence or absence of collateral flow. For example, McIntosh and Huang found p-aminohippurate (PAH) transport differed in tissue slices from rat kidneys made ischemic by surgical techniques designed to include capsular and ureteral blood vessels as compared to those from kidneys in which only the main renal artery was acutely occluded. Reimer and Jennings found differences in PAH accumulation between slices from kidneys made ischemic by removal from the rats as contrasted to acute arterial occlusion. Since we find no evidence for collateral flow to renal parenchyma during acute arterial occlusion, explanation of these biochemical differences in terms of persistent, functionally significant blood flow during occlusion of the main renal artery is untenable. Differences in collateral circulation in dogs also have been proposed by Randall to explain differences in the biochemical effects of ischemia induced by renal arterial versus renal pedicle occlusion. Since recent studies from our laboratory using microspheres in the dog indicate no significant collateral flow during acute arterial occlusion, this explanation appears untenable in this species, as well as in the rat. It should be noted that there is convincing evidence that a functionally significant circulation capable of maintaining viable parenchyma may develop during chronic partial occlusion of the main renal artery in the dog. In conclusion, nutrient blood flow to renal parenchyma is negligible hours or weeks after complete occlusion of the renal artery in the rat, despite the anatomic presence of numerous collateral vessels.

TABLE 1 Renal Blood Flow

<table>
<thead>
<tr>
<th>Time of occlusion*</th>
<th>Radioactivity per kidney (counts/min)</th>
<th>RBF, ischemic/ control (%)</th>
<th>RBF (ml/min)</th>
<th>Kidney weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ischemic</td>
<td>Control</td>
<td>Ischemic</td>
<td>Control</td>
</tr>
<tr>
<td>Acute (4)</td>
<td>54 ± 12†</td>
<td>17458 ± 1985</td>
<td>0.34 ± 0.10</td>
<td>0.03 ± 0.01†</td>
</tr>
<tr>
<td>Early chronic (4)</td>
<td>466 ± 162†</td>
<td>16017 ± 4145</td>
<td>2.80 ± 0.62</td>
<td>0.29 ± 0.08†</td>
</tr>
<tr>
<td>Late chronic (7)</td>
<td>233 ± 86†</td>
<td>22929 ± 3776</td>
<td>1.03 ± 0.37</td>
<td>0.12 ± 0.03†</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

* Acute = 1 hour after arterial occlusion; early chronic = 5-12 days; late chronic = 28-60 days. Numbers in parentheses = number of rats studied.
† P < 0.01, ischemic vs. control.
‡ P < 0.05, ischemic vs. control.
Role of Converting Enzyme in the Responses of Rabbit Atria, Aortas, and Adrenal Zona Glomerulosa to \([\text{des-Asp}^1]\text{angiotensin I}\)

JOHN A. ACKERLY, BIE-SHUNG TSAI, AND MICHAEL J. PEACH

SUMMARY. Conversion of angiotensin I (A I) and \([\text{des-Asp}^1]\text{angiotensin I}\) (des'-A I) to angiotensin II (A II) and angiotensin III (A III), respectively, was studied in aortic strips, left atria, adrenal zona glomerulosa cell suspensions from rabbits, and with purified rabbit lung converting enzyme. Conversion of A I and des'-A I was estimated in the presence and absence of \(\beta\)-thromoparvarca nonapeptide, converting enzyme inhibitor (CEI), by measuring the changes in peptide-induced tension development in aortas and atria and on steriodogenesis in cell suspensions. The liberation of histidyl-leucine from A I and des'-A I by the enzyme preparation was studied. Angiotensin I and des'-A I possessed 23% and 1% contractile activity (aorta), and 34% and 4% positive inotropic activity (atria), respectively, when compared to A II. Inhibition of aortic and atrial converting enzymes attenuated responses to A I and des'-A I without significantly altering responses to A II and A III. The steroidogenic activity of A I and des'-A I in adrenal cells was dependent on conversion since treatment with CEI specifically abolished aldosterone biosynthesis induced by A I and des'-A I without changing the activities of A II or A III. The \(K_m\) values for A I and des'-A I determined with lung enzyme were 80 \(\mu\)M and 30 \(\mu\)M, respectively. The hydrolysis of A I and des'-A I was competitively inhibited by CEI, A II, and A III. Angiotensin III was the most potent CEI among several metabolites of A I. These results indicate that des'-A I was a better substrate than A I for isolated pulmonary converting enzyme. The present investigation clearly indicates that des'-A I is rapidly converted by purified and tissue converting enzymes. The data are consistent with the postulated alternative pathway for formation of A III from des'-A I subsequent to N-terminal degradation of A I.

THE renin-angiotensin system affects arterial blood pressure directly through the pressor effect of angiotensin II (A II, the octapeptide) and indirectly through the release of aldosterone and the subsequent retention of sodium. It generally has been accepted that A II is the only major active peptide of the renin-angiotensin system, and that metabolites of A II have little biological activity. However, angiotensin III (A III), the C-terminal heptapeptide metabolite of A II, has been shown to be a potent steriodogenic agent in the adrenal zona glomerulosa. The potential physiological significance of A III on aldosterone bio-
synthesis has been advanced by many investigators.\(^1\)\(^-\)\(^3\) Peach and Chiu\(^4\) postulated that part of the action of A II on the adrenal zona glomerulosa might be mediated through A III, which can be produced locally by the action of aminopeptidase(s) on A II. Such aminopeptidase(s) which catalyze the hydrolysis of A II have been found in various tissues.\(^5\)\(^-\)\(^6\) It was suggested that aminopeptidase(s) might similarly cleave angiotensin I (A I, the decapeptide) to form \([\text{des-Asp}^1]\text{angiotensin I}\) (des'-A I, the nonapeptide), and that A III may be generated from des'-A I by converting enzyme.\(^7\)\(^-\)\(^8\) Recently, the conversion of des'-A I to A III by partially purified porcine plasma and lung converting enzymes was reported, and des'-A I was a better substrate than A I for converting enzymes.\(^7\) The present investigation was performed using tissues from rabbits, a species known to respond to A III,\(^1\) and was undertaken to determine (1) if des'-A I was converted

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