Enhanced skeletal muscle arteriolar reactivity to angiotensin II after recovery from ischemic acute renal failure

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**Abbreviated title:** Peripheral vascular reactivity following ARF

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ABSTRACT

In addition to the long-term renal complications, previous studies suggested that post acute renal failure (ARF) rats manifest an increased pressor response to an overnight infusion of angiotensin II (Ang II). The present study tested whether recovery from ARF results in alterations in sensitivity to the peripheral vasculature. ARF was induced in Sprague-Dawley rats by 45 min of bilateral renal ischemia and reperfusion. Animals were allowed to recover renal structure and function for 5-8 weeks; after which the acute pressor responses to Ang II were evaluated either in vivo, in \textit{in situ} skeletal muscle arterioles, or in isolated gracilis muscle arteries \textit{in vitro}. Baseline arterial pressure was not different in ARF rats vs. sham-operated controls, although ARF rats exhibited an enhanced pressor response to bolus Ang II infusion versus control rats. Steady state plasma Ang II concentration and plasma renin activity were similar between ARF and control rats. Constrictor reactivity of \textit{in situ} cremasteric arterioles from ARF rats was enhanced in response to increasing concentrations of Ang II, however no difference was observed in arteriolar responses to elevated pO$_2$, norepinephrine, acetylcholine or sodium nitroprusside. Isolated gracilis muscle arteries from ARF rats also showed increased vasoconstriction in response to Ang II but not norepinephrine. In conclusion, recovery from ischemic ARF is not associated with hypertension but is associated with increased arteriolar constrictor reactivity to Ang II. While the mechanisms of this altered responsiveness are unclear, such changes may relate, in part, to cardiovascular complications in patients with ARF and/or following renal transplant.
Introduction

Ischemia/reperfusion (I/R) injury in rodents is commonly used to study acute renal failure (ARF) and ischemic injury associated with renal transplant. In rats, ARF due to I/R is reversible, and is associated with a restoration of glomerular filtration rate (GFR) and tubular morphology (19, 27). We have shown that despite the initial resolution of renal injury, that there are permanent alterations in renal structure and function such as a reduced urinary concentrating ability and a permanent reduction in peritubular capillary density (4). Despite the initial resolution of the injury, post-ischemic kidneys are prone to the development of a secondary chronic disease characterized by a gradual increase in proteinuria, advancing fibrosis, and under some conditions, and secondary decline in GFR (3, 4, 12, 31).

Delayed graft function (DGF) is a vital concern in renal transplantation and represents a significant independent predictor of graft loss (10, 11, 17, 21, 25, 29). In addition to complications with the graft, non-renal complications may also develop in the setting of renal transplant. Delayed graft function is associated with the development of post-transplant hypertension function (21) and to 38% of transplant patients die for reasons primarily attributable to cardiovascular disease, despite the presence of a functioning graft (28). Thus, it is possible that ischemic injury to kidney in the setting of transplant may predispose not only renal complications, but also may affect non-renal cardiovascular parameters.

In an earlier study, we demonstrated that following either 4 or 8 weeks of recovery from ischemic ARF, rats exhibited an increased pressor response to chronic intravenous infusion of angiotensin II (Ang II) at a dose that was subpressor in control rats (4). However, whether the increased pressor
responsiveness to Ang II was the result of alterations in the renal handling of sodium or was mediated by altered peripheral vascular responsiveness to the constrictor stimulus was not addressed. The present study investigated this previously unresolved issue and tested the hypothesis that recovery from renal ischemia/reperfusion injury is associated with an altered vascular reactivity of the peripheral circulation.

Methods

Animals and Surgical Procedures:

In all studies, male Sprague-Dawley rats weighing 275-300 g were housed with 12/12 h light/dark cycle. Animals were fed standard laboratory chow (Purina, St. Louis, MO) with 0.8% sodium content; food and water available ad libitum. Care of the rats before and during the experimental procedures was conducted in accordance with the policies of the National Institutes of Health guidelines for the care and use of laboratory animals. All protocols had received prior approval by the local Institutional Animal Care and Use Committee.

Vascular reactivity was determined in three separate groups of animals following recovery from acute renal failure. In all experiments, animals were subjected to a standard renal ischemia/reperfusion protocol (I/R) to induce ARF after 45 minutes of bilateral renal artery clamping (6, 26). After removal of the clamps, rats were allowed to recover for 5-8 weeks to allow for resolution of the ARF. In our previous study, the increased pressor response to Ang II was indistinguishable at 4 weeks and 8 weeks post ischemia (4). Following this recovery period, alterations in peripheral vascular reactivity were determined.

*Experiment 1* was designed to determine the effects of recovery from ARF on resting mean
blood pressure, plasma Ang II concentration, plasma renin activity (PRA), and the acute blood pressure response to a step-wise increase in Ang II infusion. Serum creatinine values obtained from tail-blood samples were determined 24 hours following the I/R procedures to determine the severity of the initial renal injury. Subsequent determinations of serum creatinine concentration were conducted to verify re-establishment of renal function. 7 of 8 post-ischemic animals in this experiment group recovered fully from surgery and were subjected to measurement of blood pressure and blood sampling as described below. In Experiment II, I/R injury was conducted as described in Experiment I; and 7 of 8 rats experiencing the I/R injury were utilized to determine the reactivity of *in situ* cremasteric arterioles as described below. In Experiment III, 5 of 5 rats recovered from the I/R injury; and rats from this subgroup were utilized to determine the reactivity of isolated gracilis muscle arteries as described below. All experiments included equivalent groups of sham-operated control rats, in which the renal arteries were surgically exposed in an identical fashion as for rats receiving I/R injury, but were not clamped.

**Blood sampling and Blood Pressure Measurements:**

30-31 days following the renal artery clamp or sham-operations, rats from Experiment I were instrumented with chronic indwelling vascular catheters. Rats were anesthetized with ketamine HCl (60 mg/kg), xylazine (6 mg/kg), and acepromazine maleate (0.9 mg/kg) by intraperitoneal injection. The catheters, constructed as described previously (4), were inserted into the femoral artery and vein and advanced approximately 5 cm so that the tips were in the aorta and the vena cava, distal to the renal vessels. Catheters were tunneled subcutaneously and were exteriorized at the scapula and placed inside a stainless steel spring that was secured onto the rat with stainless steel button-adaptor. Following
recovery, rats were housed individually in metabolic cages. The distal end of the catheter-spring was secured to the top of the cage to allow for unrestrained movement. Both venous and arterial catheters were filled and flushed daily with 1000 U/ml heparin in sterile saline to prevent clotting. An antibiotic solution of chloramphenicol sodium succinate (1 mg/kg) was administered daily through the venous catheter.

Rats were allowed to recover for 3 days, after which blood samples were collected for determination of plasma Ang II concentration and PRA. In order to minimize the effect of autonomic activation of PRA, blood was drawn from arterial catheters of unrestrained conscious rats. Approximately 1 ml of blood was obtained for sampling and volume replaced with sterile saline.

On day 35, rats were prepared for determination of arterial blood pressure using solid-state pressure transducers (Argon Medical Technologies, Athens, TX), which were amplified and acquired on-line using custom designed data acquisition software (Department of Physiology, Medical College of Wisconsin); pulsatile blood pressure signals were reduced to periodic (1 minute) averages of mean arterial pressure (MAP). Ang II (Sigma) was dissolved in sterile saline and administered to rats via the venous catheter using syringe infusion pump. The dose of Ang II was varied by altering the flow rate of the infusion pump from 10 – 100 µl/min. Infusion of each stepwise dose of Ang II was for 15 min. Each alteration of flow rate resulted in an increase in MAP which typically plateaued at 2-5 min and did not exhibit evidence of tachyphylaxis (data not shown); for consistency, data are presented as MAP averaged over the final 5 min. of infusion of each dose.

**Reactivity of In Situ Cremasteric Arterioles:** Animals from *Experiment II* were prepared
at 5 weeks post-I/R or sham-surgery. Rats were anesthetized with an injection of sodium pentobarbital (60 mg·kg\(^{-1}\) i.p., supplemented as necessary). All rats received tracheal intubation to facilitate maintenance of a patent airway and a carotid artery was cannulated for determination of arterial pressure immediately prior to preparation of the cremaster muscle. After the initial surgery, the cremaster muscle was prepared for television microscopy, described previously, with care taken to not disrupt the deferential feed vessels\((13, 18)\). After completion of the muscle preparation, the tissue was continuously superfused with physiological salt solution (PSS), equilibrated with a gas mixture containing 5% CO\(_2\) and 95% N\(_2\), and maintained at 35°C as it flowed over the muscle. The ionic composition of the PSS was as follows (mM): NaCl 119.0, KCl 4.7, CaCl\(_2\) 1.6, NaH\(_2\)PO\(_4\) 1.18, MgSO\(_4\) 1.17, and NaHCO\(_3\) 24.0. After an initial post-surgical equilibration period of 30 minutes, a distal arteriole of ~20 µm diameter was identified in a clearly visible region of the tissue. For the present study, arterioles had a control (no pharmacological/experimental manipulation; 0% O\(_2\) in the muscle superfusate) diameter of 20±1 µm and a maximum diameter under Ca\(^{2+}\)-free PSS containing 10\(^{-3}\)M adenosine of 32±1µm (active tone = 37±2%). Arterioles chosen for study had walls that were clearly definable, a brisk flow velocity and active tone, as indicated by the occurrence of significant dilation in response to topical application of 10\(^{-4}\)M adenosine. All arterioles that were studied were located in a region of the muscle that was away from any incision. Arteriolar diameter was determined with a video micrometer, accurate to ± 1µm.

To determine the reactivity of in situ arterioles to altered oxygen tension, the O\(_2\) content of the equilibration gas superfusing the preparation was increased from 0% to 21% O\(_2\) (5% CO\(_2\), balance N\(_2\) for
each). After ~5 minutes at the elevated oxygen level, the diameter of the arteriole under investigation was determined. Our previous studies have demonstrated that during 21% O\textsubscript{2} superfusion, the elevated superfusate pO\textsubscript{2} serves as an additional oxygen source, elevating tissue and periarteriolar pO\textsubscript{2} by approximately 30 mmHg (9, 13, 24). The reactivity of \textit{in situ} arterioles of cremaster muscle was also determined in response to increasing concentrations of: 1) norepinephrine (10\textsuperscript{-10} – 10\textsuperscript{-7} M), 2) angiotensin II (10\textsuperscript{-10} – 10\textsuperscript{-7} M), 3) acetylcholine (10\textsuperscript{-9} – 10\textsuperscript{-6} M), and 4) sodium nitroprusside (10\textsuperscript{-9} – 10\textsuperscript{-6} M).

\textbf{Assessment of Isolated Arteriolar Reactivity:} Animals from \textit{Experiment III} were prepared at 8 weeks post-recovery for evaluation of reactivity of arterioles in vitro. The small muscular branches of the femoral arteries supplying the gracilis muscle were freed from surrounding tissue and surgically removed, as described previously (32). Vessels were immediately immersed in warmed (37ºC) PSS equilibrated with 21% O\textsubscript{2}, 5% CO\textsubscript{2} and 74% N\textsubscript{2}. The PSS used in these experiments had the following ionic composition: 119.0 mM NaCl, 4.7 mM KCl, 1.6 mM CaCl\textsubscript{2}, 1.18 mM NaH\textsubscript{2}PO\textsubscript{4}, 1.17 mM MgSO\textsubscript{4}, 24.0 mM NaHCO\textsubscript{3}, 5.5 mM dextrose, and 0.03 mM ethylenediaminetetraacetic acid (EDTA).

Arteries were double cannulated with glass micropipettes and were secured to the inflow and outflow pipettes using 10-0 nylon suture in a superfusion-perfusion chamber. Vessels were then extended to their approximate \textit{in situ} length and side branches were ligated with single strands teased from 6-0 silk suture. The inflow pipette was connected to a reservoir perfusion system that allowed the intraluminal pressure and luminal gas concentration to be controlled. Vessel diameter was measured using television microscopy and an on-screen video micrometer. All vessels used in this study exhibited significant levels of active tone as evidenced by a substantial increase in resting diameter upon exposure to Ca\textsuperscript{2+}-free PSS.
After a 1 hour equilibration period, each vessel was pressurized to 80% of mean arterial pressure by clamping the outflow pipette and adjusting the height of the reservoir attached to the inflow pipette (23); and arterial reactivity was assessed in a “no-flow” condition to determine the response to angiotensin II (ANG II; $10^{-8}$ M) and norepinephrine (NE; $10^{-8}$ M). In another series of studies vessel responses to ANG II were recorded in the presence and absence the ANG II AT$_1$ receptor antagonist losartan (Merck; $10^{-6}$ M) 60 min prior to exposure with Ang II.

**Plasma renin activity and Ang II levels:** PRA was determined by measuring the conversion of angiotensinogen to angiotensin I using a modification of the method described by Sealy and Laragh (34); Angiotensin I levels were measured by RIA as described previously (33). Plasma Ang II levels were determined by RIA following HPLC separation of Ang I, Ang II and other angiotensin metabolites; the strong antibody cross-reactivity with Ang II-(2-8), Ang II-(3-8) and Ang II necessitates the HPLC separation step for accurate determination of Ang II (1-8) as described previously (33).

**Assessment of renal structure:** At termination of Experiment I and II, animals were anaesthetized with pentobarbital and a midline incision was made to expose the kidneys. Kidneys were rapidly excised, cut longitudinally and fixed by immersion in 10% buffered formalin. The tissues were prepared for routine paraffin embedding and examination by light microscopy using periodic acid-Schiff staining.

**Statistical Analyses**

All data are expressed as mean±SEM. Data were analyzed with Student’s t-test, a one-way, or repeated measures analysis of variance (ANOVA) with a Student’s Newman-Keuls post hoc test, as indicated in the appropriate figure legends; $p<0.05$ was considered to be statistically significant.
RESULTS

Acute renal failure was induced by 45 minutes of bilateral renal artery clamping and subsequent reperfusion. Evidence of ARF was indicated by serum creatinine values, which rose significantly above sham-operated values 24 hours after surgery in all experimental groups (Table 1). Within 7 days post-ischemia, serum creatinine values returned toward control values, consistent with the course of injury and recovery observed in this model. The 5-8 week time points that were the focus of the present study preceded any evidence of secondary chronic renal dysfunction as indicated by serum creatinine values (Table 1, and (4)). Urine flow rates were not measured in the current study, however, previous studies have consistently demonstrated that post-ischemic rats manifest a urinary concentrating defect at all time points of recovery (4).

Renal structure obtained from animals 5 weeks post-surgery is shown in Figure 1. Tubular morphology is generally restored throughout the renal outer medulla, as proximal tubules appear differentiated, and there little evidence of persistent tubular dilation and limited evidence of significant tubular damage. However, tubular hyperplasia and increased interstitial cellularity is observed at this time point, consistent with previous reports at this stage of recovery (35).

At five weeks of recovery, mean arterial blood pressure (MAP) was not different between ARF rats and the corresponding sham-operated control animals (Figure 2A), and baseline measurements of plasma renin activity and Ang II concentration could not be distinguished between the two animal
groups (Table 1). However, intravenous infusion of Ang II resulted in a significantly enhanced acute pressor response in post-ischemic animals vs. the response observed in corresponding sham-operated control animals (Figure 2A). Similar results were obtained when a bolus dose of Ang II (50 µg; i.v.) was administered to anesthetized rats (Figure 2B). In these studies, the response to the bolus was rapid (beginning < 10 sec) and there was no difference in the onset or duration of the response between groups (data not shown). These data suggested that rats manifest an enhanced pressor response to Ang II post-ischemic ARF.

Further exploration of this phenomenon was carried out by measuring alterations in the reactivity of in situ cremasteric arterioles to vasoactive stimuli. As presented in Figure 3, there was a dose-dependent reduction in vessel diameter in sham-operated control animals in response to Ang II exposure, while post-ischemic animals demonstrated a consistently greater vasoconstriction to Ang II (Fig 3). However, the altered reactivity of in situ skeletal muscle arterioles was specific to Ang II, as arteriolar responses to increasing concentrations of norepinephrine (Figure 4A), elevated oxygen tension (Figure 4B), or increasing concentrations of acetylcholine (Figure 4C) or sodium nitroprusside (Figure 4D) were not different between ARF rats and sham-operated control animals.

Figure 5 presents data describing the reactivity of isolated gracilis arteries from ARF and control animals. While constrictor reactivity of isolated arteries in response to $10^{-8}$M norepinephrine was comparable between the two animal groups (Panel A), the reactivity of isolated microvessels from rats following recovery from ARF was significantly increased over that determined in vessels from control animals in response to challenge with $10^{-8}$M Ang II (Panel B). This increased Ang II-induced
constrictor reactivity appeared to be mediated via the AT\(_1\) receptor subtype, as pharmacological blockade of this receptor with losartan abolished the all constrictor reactivity of vessels from both groups in response to Ang II (Panel B). Pharmacological blockade of vascular AT\(_2\) receptors (PD 123319) did not have a significant effect on the reactivity of isolated arterioles from either control rats or in animals following recovery from ARF in response to challenge with angiotensin II in (data not shown).

**Discussion**

Previous studies in our laboratory and by others have suggested that recovery from ARF is associated with a predisposition toward the development of chronic renal disease (3, 4). However, it is also possible that reversible injury to the kidney may also have effects at distant sites. Although there is currently limited information available on the long-term consequences of ARF in native kidneys; it is clear that renal function is not always restored completely in surviving individuals following ARF, as this syndrome can be associated with a secondary decline in renal function (1, 7, 8, 10).

It is possible that acute ischemic renal injury may also influence the function of sites outside of the kidney. For example, DGF in the setting of renal transplant is a predisposes hypertension (10, 11, 17, 21, 25, 29). Moreover, it was recently reported that 38% of all deaths following kidney transplant occur with a functioning graft. The largest proportion of these deaths was attributable to cardiovascular disease and correlated to a number of significant risk factors including delayed graft function and acute rejection (28). While these observations suggest that renal ischemic injury may affect extra-renal vascular parameters, we are not aware of any studies using animals models that have been geared
toward evaluating long term extra-renal vascular function in animals models.

In a previous study (4), we demonstrated that post-ischemic animals (4 and 8 weeks) manifested increased responsiveness to an overnight infusion of Ang II. That study did not examine whether the response was due altered renal handling of sodium, or rather to acute alterations in vascular sensitivity to Ang II. Data from the current study suggest that increased Ang II-induced pressor reactivity is at least partially dependent on alterations to the sensitivity of the peripheral vascular networks since: 1) there is a significant increase in the acute pressor response to Ang II infusion and 2) stimulation of both in situ cremaster muscle arterioles and isolated gracilis muscle arteries result in a significant increase in the constrictor response to challenge with Ang II. The possibility that the post-ischemic kidney may also manifest alterations in Na handling remains an important issue that was not addressed in the current study.

Several major questions remain for further understanding of the observations in the current study, for example; what is the nature of the altered signaling mechanisms underlying of the enhanced peripheral vascular reactivity to Ang II? It is curious that altered responsiveness was seen in response to Ang II but not to norepinephrine. Previously, Weber et al. (36), also demonstrated an enhanced constriction of isolated gracilis arteries in response to challenge with Ang II, but not norepinephrine when normal rats were placed on a chronic high salt (4% NaCl) diet, although this increased constrictor reactivity was not exhibited within the cremasteric microcirculation. It has been suggested that suppression of circulating plasma Ang II levels on high salt diet may upregulate AT₁ receptors on the vasculature, leading to increased responsiveness (37). However, in the current study, there was no
consistent alteration in the plasma levels of Ang II or PRA in the post-ischemic group vs. the sham-operated control group. Although we have thus far been unable to reliably assess AT\(_1\) receptor levels in dissected skeletal muscle microvessels, the possibility that AT\(_1\) receptors may be enhanced in peripheral vasculature during the recovery from ARF represents a possibility worthy of further consideration. It is also possible that AT\(_1\) post-receptor signaling might be altered following ischemic injury.

A second important question of interest is what are the signals that arise from the injured kidney following I/R and how do these signals cause an altered vascular reactivity in the periphery? In other models of renal disease (e.g., 5/6 nephrectomy), uremia and/or the presence of hypertension have been implicated in affecting vascular reactivity (14, 23). However, in the present study, rats following recovery from ARF were not uremic and were normotensive when the alterations in peripheral sensitivity to Ang II were evaluated.

It is logical to speculate that the injured kidney releases circulating factors that modulate peripheral responsiveness to Ang II. There are clear alterations in the humoral milieu following ischemic injury, notably alterations in inflammatory cytokines and endothelin-1 have been reported (15, 16, 20). Speculation that altered cytokine status following IR might mediate extrarenal effects is supported by the observation of Kelly that circulating TNF-a and IL-1 affected cardiac function and apoptosis (22). However, most studies to date have focused on early injury periods, and the cytokine/humoral profile of rats after recovery from ARF has not been carefully investigated. Recent data from our laboratory using microarray analysis, as well as the work of many other investigators suggest that the post-ischemic kidney may still be in a pro-inflammatory state even after its apparent recovery (5). In addition, a study
by Azuma et al., demonstrated the late up-regulation of the renal expression of TNF-a, interleukin-1, and iNOS (2). Whether persistent inflammation present in the post-ischemic recovered kidney is associated with the altered peripheral vascular sensitivity reported here is unclear and requires further investigation.

A final consideration is whether the altered responsiveness to Ang II reported here in skeletal muscle vascular beds is also manifested in other vascular beds. Indeed, the possibility that the renal vasculature may be more sensitive to Ang II stimulation has not been addressed. This possibility might relate to the observation by Patgulan et al., in which AT1 antagonism attenuated the development of chronic renal disease following an initial recovery from I/R (30). It might also be worthy to investigate alterations in the coronary or cerebral circulations following recovery from I/R, since myocardial infarction and stroke are among the leading causes of death with a functioning graft (28).

In conclusion, a common rat model of reversible ischemic ARF is associated with an initial recovery response, but persistent alterations in physiological state. When maintained on standard laboratory chow, hypertension is not observed, however, increased pressor responsiveness is observed to Ang II. This increased responsiveness is observed in microvessels and resistance arteries of skeletal muscle vascular beds. The mechanism of the increased responsiveness and its potential relationship to cardiovascular complications in the setting of renal I/R in the clinical setting remain to be determined.
ACKNOWLEDGMENTS

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REFERENCES


FIGURE LEGENDS

Figure 1. Representative histological images following recovery from ischemic ARF from rats in Experiment I. Shown are PAS-stained sections through renal outer medulla of a sham-operated rat (A), and a post-ischemic rat (B). Renal tubular structure is largely restored in post-ischemic animals following 35 days of recovery from ARF. However, tubular hypercellularity and increased interstitial cellularity is evident (open arrow). Isolated areas of persistent injury are occasionally observed (dark arrow). Magnification is shown.

Figure 2. Acute pressor response to Ang II in rats following 5 weeks recovery from ischemia/reperfusion injury. A; results from Experiment I obtained in conscious unrestrained animals. Male Sprague-Dawley rats were instrumented for evaluation of blood pressure response to Ang II at Day 35 post-ischemia. * indicates P < 0.05 post-ischemic recovered animals vs. sham-operated controls by Student’s t-test. Concentration. B; results from Experiment II obtained in anesthetized animals to intravenous bolus of Ang II. Results are the measured peak of MAP following bolus injection. * indicates P < 0.05 post-ischemic recovered animals vs. sham-operated controls by Student’s t-test.

Figure 3. Skeletal muscle arteriolar reactivity to Ang II following recovery from ischemia/reperfusion injury. The reactivity of 3rd order arterioles was determined in animals 35 days following recovery from either sham-surgery or ischemia/reperfusion (from Experiment II), in the in
situ cremaster muscle. The reactivity to increasing doses of Ang II were determined and the response between groups was evaluated by comparing differences in the slope of the dose response curve (β-coefficient). † indicates β-coefficient is significantly different in post-ischemic rats vs. sham-operated animals as determined by one-way ANOVA and Student’s Newman-Keuls post hoc test, P < 0.05.

Figure 4. Skeletal muscle arteriolar reactivity in response to other vasodilators or vasoconstrictors is unaltered following ischemia/reperfusion injury. The reactivity of 3rd order arterioles was determined in animals 5 weeks following I/R injury using the in situ cremaster muscle as in Figure 3. The constrictor response to altering % O2 (A) and norepinephrine (B) was determined at the indicated concentrations. The vasodilatory response of acetylcholine and sodium nitroprusside is shown in C and D, respectively. There was no difference in the β-coefficient value between the sham-operated and post-ischemic group.

Figure 5. Differential reactivity of angiotensin II in isolated gracilis arteries from sham-operated or post-ischemic animals. Gracilis arteries from post-ischemic or sham-operated animals were microdissected from rats following sham-surgery or following recovery from I/R injury (experiment III). The reactivity of gracilis arteries was determined in vitro as described in Methods. A) The
vasoconstrictor response to $1 \times 10^{-8}$ norepinephrine is shown. * indicates $P < 0.05$ vs. prestimulation values, by paired Students t-test. NS, indicates not statistically different between NE stimulated values in vessels obtained from sham-operated or post-ischemic animals. B) The vasoconstrictor response to Ang II ($1 \times 10^{-8}$) was determined in gracilis arteries from sham-operated and post-ischemic animals. * indicates $P < 0.05$ vs. prestimulation values by paired Students t-test; note the similar magnitude of constriction in vessels from sham-operated animals to value obtained in panel A and panel B. † indicates $P < 0.05$ in vessels from post-ischemic animals vs. corresponding sham-operated control animals. ‡ indicates $P < 0.05$ in losartan-treated vessels vs. Ang II stimulated vessels without losartan.
Table 1. Renal function, plasma angiotensin II and renin activity of rats from the 3 experimental groups in response to renal ischemia/reperfusion injury.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Scr mg/dl 24 hours post-surgery</th>
<th>Scr mg/dl at termination&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Plasma Ang II pg/ml 34 days postsurgery</th>
<th>PRA ng/mg/hour 34 days postsurgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Exp I</td>
<td>5</td>
<td>0.5±0.05</td>
<td>0.4± 0.1</td>
<td>13.2 ± 3.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARF Exp I</td>
<td>7</td>
<td>3.2±0.2*</td>
<td>0.4± 0.1</td>
<td>8.6 ± 2.2(P=0.22)</td>
<td>2.4 ± 0.4(P=0.09)</td>
</tr>
<tr>
<td>Sham Exp II</td>
<td>4</td>
<td>0.4±0.02</td>
<td>0.4±0.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ARF Exp II</td>
<td>7</td>
<td>3.6±0.2*</td>
<td>0.6±0.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sham Exp III</td>
<td>5</td>
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</tr>
<tr>
<td>ARF Exp III</td>
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<td>3.6±0.2*</td>
<td>0.6±0.1</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* indicates serum creatinine significantly different from sham-operated controls within each experiment, P < 0.05 by Student’s t-test. <sup>a</sup>Termination of Experiment I and Experiment II was at 5 weeks postsurgery, and termination of Experiment III was at 8 weeks postsurgery.  
indicates serum creatinine post-ischemic animals vs. sham-operated controls.  
<sup>b</sup>Plasma levels for angiotensin II and PRA in sham-operated group based on N=3. ND, plasma Ang II and PRA were not determined in Experiments II and III, due to the experimental design.
Basile et al, Figure 1
Basile et al, Figure 2
log Angiotensin II Concentration (M)

Arteriolar Diameter (µm)

- Sham; $\beta = -0.633 \pm 0.085$
- ARF; $\beta = -1.052 \pm 0.076$†

Basile et al., Figure 3
Basile et al., Figure 4