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

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1Department of Cellular and Integrative Physiology, Indiana University, Indianapolis, Indiana; 2Department of Physiology, Medical College of Wisconsin, Milwaukee Wisconsin; and 3Center for Interdisciplinary Research in Cardiovascular Sciences, Department of Physiology and Pharmacology, West Virginia University School of Medicine, Morgantown, West Virginia

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Basile, David P., Deborah L. Donohoe, Shane A. Phillips, and Jefferson C. Frisbee. Enhanced skeletal muscle arteriolar reactivity to ANG II after recovery from ischemic acute renal failure. Am J Physiol Regul Integr Comp Physiol 289: R1770–R1776, 2005. First published August 11, 2005; doi:10.1152/ajpregu.00269.2005.—In addition to the long-term renal complications, previous studies suggested that after acute renal failure (ARF), rats manifest an increased pressor response to an overnight infusion of 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 wk, after which the acute pressor responses to ANG II were determined. After this recovery period, alterations in peripheral vascular reactivity were determined. In all studies, male Sprague-Dawley rats weighing 275–300 g were housed with a 12:12-h light-dark cycle. Animals were fed standard laboratory chow (Purina, St. Louis, MO) with 0.8% sodium content; food and water were 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 received prior approval from the local Institutional Animal Care and Use Committee.

Vascular reactivity was determined in three separate groups of animals after recovery from ARF. In all experiments, animals were subjected to a standard renal I/R protocol to induce ARF after 45 min of bilateral renal artery clamping (6, 26). After removal of the clamps, rats were allowed to recover for 5–8 wk to allow for resolution of the ARF. In our group’s previous study (4), the increased pressor response to ANG II was indistinguishable at 4 and 8 wk postischemia. After 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 concent-
Conducted as described for Experiment I blood sampling, as described below. In surgery and were subjected to measurement of blood pressure and conducted to verify reestablishment of renal function. Seven of eight Subsequent determinations of serum creatinine concentration were the I/R procedures to determine the severity of the initial renal injury. Values obtained from tail blood samples were determined 24 h after response to a stepwise increase in ANG II infusion. Serum creatinine Renal function, plasma ANG II, and renin activity of rats from Experiments I–III.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>SCr, mg/dl 24h postsurgery</th>
<th>SCr, mg/dl at termination</th>
<th>Plasma ANG II, pg/ml 34 days postsurgery</th>
<th>PRA, ng·mg⁻¹·h⁻¹ 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†</td>
<td>1.2±0.6†</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>
<td>0.4±0.02</td>
<td>0.4±0.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ARF Exp III</td>
<td>5</td>
<td>3.6±0.2*</td>
<td>0.6±0.1</td>
<td>ND</td>
<td>ND</td>
</tr>
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Values are means ± SE; n = no. of rats. ARF, acute renal failure; Exp, experiment; SCr, serum creatinine; PRA, plasma renin activity. Termination of Experiments I and II was at 5 wk postsurgery, and termination of Experiment III was at 8 wk postsurgery. *P < 0.05, serum creatinine postischemic animals vs. sham-operated controls. †Plasma levels for ANG II and PRA in sham-operated group are based on n = 3. ND, plasma ANG II and PRA levels were not determined in Experiments II and III due to the experimental design.

Blood sampling and blood pressure measurements. Thirty to thirty-one days after 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 ~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. After 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 1,000 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. To minimize the effect of autonomic activation of PRA, we drew blood from arterial catheters of unrestrained conscious rats. Approximately 1 ml of blood was obtained for sampling and the volume replaced with sterile saline.

On day 35, rats were prepared for determination of arterial blood pressure with solid-state pressure transducers (Argon Medical Technologies, Athens, TX), which were amplified and acquired online by using custom-designed data acquisition software (Department of Physiology, Medical College of Wisconsin); pulsatile blood pressure signals were reduced to periodic (1 min) averages of mean arterial pressure (MAP). ANG II (Sigma) was dissolved in sterile saline and administered to rats via the venous catheter by using a syringe infusion pump. The dose of ANG II was varied by altering the flow rate of the infusion pump from 10 to 100 μl/min. Infusion of each stepwise dose of ANG II lasted for 15 min. Each alteration of flow rate resulted in an increase in MAP that 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 wk post-I/R or sham surgery. Rats were anesthetized with an injection of pentobarbital sodium (60 mg/kg ip, supplemented as necessary). All rats received tracheal intubation to

Fig. 1. Representative histological images after recovery from ischemic acute renal failure (ARF) from rats in Experiment I. Shown are periodic acid-Schiff (PAS)-stained sections through renal outer medulla of a sham-operated rat (A) and a postischemic rat (B). Renal tubular structure is largely restored in postischemic animals after 35 days of recovery from ARF. However, tubular hypercellularity and increased interstitial cellularity are evident (open arrows). Isolated areas of persistent injury are occasionally observed (brown arrow).
facilitate maintenance of a patent airway, and a carotid artery was cannulated for determination of arterial pressure immediately before preparation of the cremaster muscle. After the initial surgery, the cremaster muscle was prepared for television microscopy, described previously (13, 18), with care taken to not disrupt the deferential feed vessels. After completion of the muscle preparation, the tissue was continuously superfused with physiological salt solution (PSS) equilibrated with a gas mixture containing 5% CO₂ and 95% N₂ and maintained at 35°C as it flowed over the muscle. The ionic composition of the PSS was as follows (in mM): 119.0 NaCl, 4.7 KCl, 1.6 CaCl₂, 1.18 NaH₂PO₄, 1.17 MgSO₄, and 24.0 NaHCO₃. After an initial postsurgical equilibration period of 30 min, a distal arteriole of ~20 μm in diameter was identified in a clearly visible region of the tissue. For the present study, arterioles had a control (no pharmacological/experimental manipulation; 0% O₂ in the muscle superfusate) diameter of 20 ± 1 μm and a maximum diameter under Ca²⁺-free PSS containing 10⁻⁴ M adenosine of 32 ± 1 μm (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 ±1 μm.

To determine the reactivity of in situ arterioles to altered oxygen tension, we increased the O₂ content of the equilibration gas superfusing the preparation from 0 to 21% O₂ (5% CO₂, balance N₂ for each). After ~5 min at the elevated oxygen level, the diameter of the arteriole under investigation was determined. Our previous studies have demonstrated that during 21% O₂ superfusion, the elevated superfusate PO₂ serves as an additional oxygen source, elevating tissue and periarteriolar PO₂ by ~30 mmHg (9, 13, 24). The reactivity of in situ arterioles of cremaster muscle was also determined in response to increasing concentrations of 1 norepinephrine (10⁻⁷–10⁻⁴ M), 2 ANG II (10⁻⁷–10⁻⁴ M), 3 ACh (10⁻⁵–10⁻⁶ M), and 4 sodium nitroprusside (10⁻⁷–10⁻⁴ M).

Assessment of isolated arteriolar reactivity. Animals from Experiment III were prepared at 8 wk after 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 (37°C) PSS equilibrated with 21% O₂, 5% CO₂, and 74% N₂. The PSS used in these experiments had the following ionic composition (in mM): 119.0 NaCl, 4.7 KCl, 1.6 CaCl₂, 1.18 NaH₂PO₄, 1.17 MgSO₄, 24.0 NaHCO₃, 5.5 dextrose, and 0.03 EDTA.

Arteries were double cannulated with glass micropipettes and were secured to the inflow and outflow pipettes with 10-0 nylon suture in a superfusion-perfusion chamber. Vessels were then extended to their approximate 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 Ca²⁺-free PSS. After a 1-h equilibration period, each vessel was pressurized to 80% of MAP by clamping the outflow pipette and adjusting the height of the reservoir attached to the inflow pipette (23), and arteriolar reactivity was assessed in a “no-flow” condition to determine the response to ANG II (10⁻⁸ M) and noradrenaline (10⁻⁸ M). In another series of studies, vessel responses to ANG II were recorded in the presence and absence of the ANG II AT₁ receptor antagonist losartan (Merck; 10⁻⁶ M) 60 min before exposure to ANG II.

PRA and ANG II levels. PRA was determined by measuring the conversion of angiotensinogen to ANG I by using a modification of the method described by Sealey and Laragh (34); ANG I levels were measured using RIA, as described previously (33). Plasma ANG II levels were determined using RIA after HPLC separation of ANG I, ANG II, and other angiotensin metabolites; the strong antibody

Fig. 2. Acute pressor response to ANG II in rats after 5 wk of recovery from ischemia/reperfusion (I/R) 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 postischemia. *P < 0.05, postischemic recovered animals vs. sham-operated controls (Student’s t-test). B: results from Experiment II obtained in anesthetized animals in response to intravenous bolus injection of ANG II. Results are the measured peak of mean arterial blood pressure (ΔMAP) after bolus injection. *P < 0.05, postischemic recovered animals vs. sham-operated controls (Student’s t-test).

Fig. 3. Skeletal muscle arteriolar reactivity to ANG II after recovery from I/R injury. The reactivity of third-order arterioles was determined in animals 35 days after recovery from either sham surgery or I/R (from Experiment II) in the in situ cremaster muscle. The reactivity to increasing doses of ANG II was determined, and the response between groups was evaluated by comparing differences in the slope of the dose-response curve (β-coefficient). *P < 0.05, β-coefficient is significantly different in postischemic rats vs. sham-operated animals (1-way ANOVA and Student-Newman-Keuls post hoc test).
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 Experiments I and II, animals were anesthetized with pentobarbital sodium 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 means ± SE. Data were analyzed with Student’s t-test or a one-way or repeated-measures ANOVA with a Student-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 min 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 h after surgery in all experimental groups (Table 1). Within 7 days posts ischemia, serum creatinine values returned toward control values, consistent with the course of injury and recovery observed in this model. The 5- to 8-wk 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 Ref. 4). Urine flow rates were not measured in the current study; however, previous studies have consistently demonstrated that postischemic rats manifest a urinary concentrating defect at all time points of recovery (4).

Renal structure obtained from animals 5 wk postsurgery is shown in Fig. 1. Tubular morphology is generally restored throughout the renal outer medulla, because proximal tubules appear differentiated, and there is 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 5 wk of recovery, MAP was not different between ARF rats and the corresponding sham-operated control animals (Fig. 2A), and baseline measurements of plasma renin activity and

![Image](https://api.imgur.com/IQ.png)
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 postischemic animals compared with the response observed in corresponding sham-operated control animals (Fig. 2A). Similar results were obtained when a bolus dose of ANG II (50 μg iv) was administered to anesthetized rats (Fig. 2B). In these studies, the response to the bolus was rapid (beginning in <10 s), and there was no difference in the onset or duration of the response between groups (data not shown). These data suggest that rats manifest an enhanced pressor response to ANG II after 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 Fig. 3, there was a dose-dependent reduction in vessel diameter in sham-operated control animals in response to ANG II exposure, whereas postischemic animals demonstrated a consistently greater vasoconstriction to ANG II. However, the altered reactivity of in situ skeletal muscle arterioles was specific to ANG II, because arteriolar responses to increasing concentrations of norepinephrine (Fig. 4A), elevated oxygen tension (Fig. 4B), or increasing concentrations of ACh (Fig. 4C) or sodium nitroprusside (Fig. 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. Whereas the constrictor reactivity of isolated arteries in response to 10⁻⁸ M norepinephrine was comparable between the two animal groups (Fig. 5A), the reactivity of isolated microvessels from rats after recovery from ARF was significantly increased over that determined in vessels from control animals in response to challenge with 10⁻⁸ M ANG II (Fig. 5B). This increased ANG II-induced constrictor reactivity appeared to be mediated via the AT₁ receptor subtype, because pharmacological blockade of this receptor with losartan abolished all the constrictor reactivity of vessels from both groups in response to ANG II (Fig. 5B). Pharmacological blockade of vascular AT₂ receptors (PD-123319) did not have a significant effect on the reactivity of isolated arterioles from either control animals or rats after recovery from ARF in response to challenge with ANG II (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 also is possible that reversible injury to the kidney also may 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 after ARF, because 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 predisposes hypertension (10, 17, 21, 25, 29, 31a). 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 DGF and acute rejection (28). Although these observations suggest that renal ischemic injury may affect extrarenal vascular parameters, we are not aware of any studies that have been geared toward evaluating long-term extrarenal vascular function in animal models.

In a previous study (4), our group demonstrated that postischemic animals (4 and 8 wk) manifested increased responsive-ness to an overnight infusion of ANG II. That study did not examine whether the response was due to 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, because I) 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 results in a significant increase in the
constrictor response to challenge with ANG II. The possibility that the postischemic kidney may also manifest alterations in sodium 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 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 a 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 postischemic group compared with the sham-operated control group. Although we have thus far been unable to reliably assess AT1 receptor levels in dissected skeletal muscle microvessels, the possibility that AT1 receptors may be enhanced in peripheral vasculature during the recovery from ARF represents a possibility worthy of further consideration. It is also possible that AT1 postreceptor signaling might be altered after ischemic injury.

A second important question of interest is what are the signals that arise from the injured kidney after 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 has been implicated in affecting vascular reactivity (14, 23). However, in the present study, rats after 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 after ischemic injury; notably, alterations in inflammatory cytokines and endothelin-1 have been reported (15, 16, 20). Speculation that an altered cytokine status after I/R might mediate extra-renal effects is supported by the observation by Kelly (22) that circulating TNF-α and IL-1 affected cardiac function and apoptosis. 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 (5), as well as the work of many other investigators, suggest that the postischemic kidney may still be in a proinflammatory state even after its apparent recovery. In addition, a study by Azuma et al. (2) demonstrated the late upregulation of the renal expression of TNF-α, IL-1, and inducible nitric oxide synthase. Whether persistent inflammation present in the postischemic recovered kidney is associated with the altered peripheral vascular sensitivity reported in the present study is unclear and requires further investigation.

A final consideration is whether the altered responsiveness to ANG II reported in the present study 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 Patgulalan et al. (30) that AT1 antagonism attenuated the development of chronic renal disease after an initial recovery from I/R. It may also be worthy to investigate alterations in the coronary or cerebral circulation after recovery from I/R, because 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 rats were maintained on standard laboratory chow, hypertension was not observed; however, increased pressor responsiveness to ANG II was observed. This increased responsiveness was 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 remains to be determined.

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REFERENCES