BASIC RESEARCH STUDIES

From the Southern Association for Vascular Surgery

Suprarenal aortic clamping and reperfusion decreases medullary and cortical blood flow by decreased endogenous renal nitric oxide and PGE_2 synthesis

Stuart I. Myers, MD, FACS, Li Wang, BS, Fang Liu, BS, and **Lori L. Bartula, BS,** *Richmond, VA*

Objective: **This study examined the hypothesis that clamping the aorta above the superior mesenteric artery (SMA) followed by suprarenal aortic clamping and reperfusion (SRACR) decreases microvascular blood flow by loss of endogenous medullary and cortical nitric oxide (NO) and prostaglandin (PG) E_2 synthesis.**

Study Design: **Anesthetized male Sprague-Dawley rats (350 g) had either microdialysis probes or laser Doppler fibers inserted into the renal cortex to a depth of 2 mm and into the renal medulla at 4 mm. Laser Doppler blood flow was continuously monitored (data reported as percentage of change compared to basal), and the microdialysis probes were connected to a syringe pump and perfused in vivo at 3 μL/min with lactated Ringer solution. Dialysate fluid was collected at basal time zero, following 30 minutes of suprarenal aortic clamping (ischemia) followed by 60 minutes of reperfusion and compared to a sham operation. Both groups were treated with saline carrier, indomethacin (INDO) (10 mg/kg, a cyclooxygenase [COX] inhibitor), N^G-nitro-L-arginine methyl ester (L-NAME) (20 mg/kg, a NO synthase [NOS] inhibitor), or L-arginine (200 mg/kg, an NO precursor). Dialysate was analyzed for total NO (μM) and PGE_2 (pg/mL) synthesis. The renal cortex and medulla were analyzed for inducible NOS (iNOS) and COX-2 content by Western blot. All data are reported as mean ± SEM, N > 5 and analyzed by analysis of variance.**

Results: **SRACR caused a marked decrease in medullary and cortical blood flow with a concomitant decrease in endogenous medullary and cortical NO synthesis. Treatment with L-NAME further decreased blood flow and NO synthesis in the medulla and cortex. L-Arginine restored medullary and cortical NO synthesis and blood flow in the cortex but not the medulla. SRACR did not alter renal medullary or cortical PGE_2; however, addition of INDO, COX inhibitor, caused a concomitant decrease in medullary and cortical PGE_2 synthesis and blood flow.**

Conclusions: **NO is an important endogenous renal vasodilator that, when maintained can help preserve cortical blood flow following SRACR. These data also suggest that avoidance of COX-2 inhibitors can help maintain endogenous renal cortical and medullary PGE_2 synthesis and thus contribute to maintaining normal blood flow. (J Vasc Surg 2005;42:524-31.)**

Clinical Relevance: **This study is the first to combine in vivo physiologic assays to simultaneously identify clinically relevant intrarenal vasodilators (cortical and medullary) that are required to maintain microvascular blood flow. Identification of endogenous renal cortical and medullary vasodilators responsible for maintaining renal microvascular blood flow will allow development of treatment strategies to preserve these vasodilators following SRACR. Successful preservation of endogenous intrarenal vasodilators will help maintain renal microvascular blood flow and renal function in the treatment of complex aortic pathology that requires SRACR.**

Over the past 30 years, considerable progress has been made in the management of patients undergoing suprarenal aortic reconstructive surgery. Despite these advances, renal dysfunction continues to be a frequent complication of suprarenal aortic reconstruction.[1-3] The pathophysiology of renal azotemia following clamping of the aorta above the superior mesenteric artery (SMA) followed by suprarenal aortic clamping and reperfusion (SRACR) is not well understood. Several animal models have shown decreases in renal blood flow, glomerular filtration rate (GFR), urine flow, and fractional sodium reabsorption and an increase in renal vascular resistance following SRACR.[4-6] The mechanisms of these alterations in renal function following SRACR are not known. Nitric oxide (NO)[7-13] and prostaglandin (PG) E_2 and PGI_2 (vasodilator eicosanoids)[14-18] have been proposed as important endoge-

From the McGuire Research Institute/McGuire VA Medical Center and Department of Surgery, Virginia Commonwealth University.

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Reprint requests: Stuart I. Myers, MD, FACS, Department of Surgery, Virginia Commonwealth University, Ninth Floor, West Hospital, 1200 E. Broad Street, Richmond, VA 23298-0108 (e-mail: simyers@vcu.edu).

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nous mediators that contribute to arterial dilatation of the renal microvascular bed and maintenance of renal blood flow. Loss of endogenous NO or vasodilator eicosanoids have been associated with renal vasoconstriction following infrarenal aortic clamping and reperfusion and renal ischemia/reperfusion injury.[19-26]

There is no literature regarding the effects of SRACR on regional blood flow or endogenous renal eicosanoid and NO synthesis within the kidney. This study examined the hypothesis that SRACR decreases microvascular blood flow by loss of endogenous medullary and cortical NO and PGE_2 synthesis.

MATERIAL AND METHODS

Animal model. All animal surgery and care were performed using the established guidelines approved by the Institutional Animal Care and Use Committee, Virginia Commonwealth University/Medical College of Virginia and the McGuire VA Medical Center, Richmond, Va. Male Sprague-Dawley rats (350 g; Harlan, Indianapolis, Ind) were anesthetized with 2% isoflurane vaporized (oxygenated) by oxygen. The rats were placed on a small animal operating table with fixed bar clamps for precise placement of microdialysis catheters or placement of micropositioners for two laser fiber probes. The right femoral artery was cannulated, heparinized (100 U/kg), and connected to a blood pressure analyzer (BPA; Digimed, Louisville, Ky). Perivascular ultrasonic blood flow probes (Transonic Systems, Inc., Ithaca, NY) were placed around the renal artery, and the abdominal aorta and data were collected as milliliters per minute. Body temperature was monitored via rectal temperature probe. The animal's abdomen was filled with saline and lubricating jelly to insulate contact between blood flow probes and blood vessels. Both the microdialysis group and the laser Doppler group were treated with saline carrier, indomethacin (10 mg/kg, a cyclooxygenase [COX] inhibitor), L-NAME (N^G-nitro-L-arginine methyl ester) (20 mg/kg, an NO synthase [NOS] inhibitor), or L-arginine (200 mg/kg, an NO precursor).[21,27] The renal cortex and medulla from the left kidney were separated and analyzed for inducible NOS (iNOS) and COX-2 content by Western blot (see below).

Microdialysis probes in vivo perfusion. Microdialysis probes were prepared in advance to allow curing of the epoxy and glue used to connect segments of PE-50 tubing, PE-10 tubing (Becton Dickinson, Sparks, Md), flexible fused silica tubing (Polymicro Technologies, Phoenix, Ariz), microdialysis tubing (13-kd transmembrane diffusion cutoff tubing) (Spectrum Laboratories, Rancho Dominquez, Calif), and additional flexible fused silica tubing.[28-30] One microdialysis probe was inserted into the renal cortex to a depth of 2 mm and another into the renal medulla at 4 mm. These probes were connected to a syringe pump and perfused at a rate of 3 L/min with lactated Ringer solution. Dialysate fluid was collected at basal time zero, following 30 minutes of suprarenal aortic clamping (ischemia) followed by 60 minutes of reperfusion and compared to a sham (SH) operation. Dialysate samples were collected and frozen at −80°C until assayed for NO and PGE_2. Use of the microdialysis probes allowed NO and PGE_2 to diffuse in and out of the dialysis probe depending on the local concentration. As NO and PGE_2 are not stored within the cell, release of these substances represent de novo synthesis. After completion of each experiment, the kidneys were reopened to confirm accurate placement of the microdialysis probes.

Laser Doppler renal cortical and medullary blood flow analysis. The right kidney was isolated and placed in a 21-mm Lucite kidney cup. Ultrasonic blood flow probes (Transonic Systems, Inc.) were placed around the renal artery and abdominal aorta, and data were collected in milliliters per minute. Body temperature was monitored via rectal probe. Micropositioners were used to insert the shallow (2 mm cortical) and deep (4 mm medullary) calibrated laser fiber probes. The position of the fibers within the kidneys was checked after each experiment. A Periflux 4001 Dual Channel Laser Doppler flowmeter (Perimed, Järfälla, Sweden) was used to evaluate microvascular perfusion. Measurements were expressed as arbitrary perfusion units. The perfusion units represent the product of the velocity and the concentration of moving blood cells within the measuring volume. After a 5-minute equilibration period, basal readings were recorded and subsequent readings recorded every 10 minutes throughout the ischemia/reperfusion and sham procedures.[22-25]

Enzyme immunoassays.[19] The renal microdialysis effluent was frozen at −80°C until assayed for PGE_2 by an immunoassay kit (Cayman Chemical, Ann Arbor, Mich). Samples were run in duplicate and reported as picograms of PGE_2 per milliliter (mean ± SEM).[31]

NO analysis. A Sievers 280 NOA Nitric Oxide Analyzer with Radical Purger (Sievers Instruments, Boulder, Colo) was used to detect NO (μM) and its reaction products in biologic samples. The technique used for the measurement of NO and its oxidation products was the reduction of nitrate, nitrite, and nitrosothiols using vanadium (III) and hydrochloric acid at 90°C. Data are reported as μM.

Protein assays. The kidneys were harvested and placed on ice. Cortical and medullary tissue were rapidly separated, frozen in liquid nitrogen, and stored at −80°C until assayed for protein. Total protein was determined by the Bradford method[31] using the Bio-Rad protein assay dye reagent concentrate (Bio-Rad Laboratories, Hercules, Calif). A 5X SDS sample buffer (1X = 62.5 mmol/L Tris-HCl, pH 8.0; 2% sodium dodecyl-sulfate (SDS); 0.025% bromophenol blue; 20% glycerol; 5% β-mercaptoethanol) was added to the protein samples and stored at −80°C until assayed by Western blot.

SDS-polyacrylamide gel electrophoresis/Western blot analyses. Protein samples diluted in SDS sample buffer were solubilized by boiling for 4 minutes. Aliquots (25 μg) were loaded into gel lanes of a vertical electrophoretic unit (Bio-Rad), and protein separation by molecular weight was carried out on 1-mm 7% polyacrylamide resolving gel with a 1-mm 3% stacking gel by the Laemmli method,[32] which includes prestained mo-

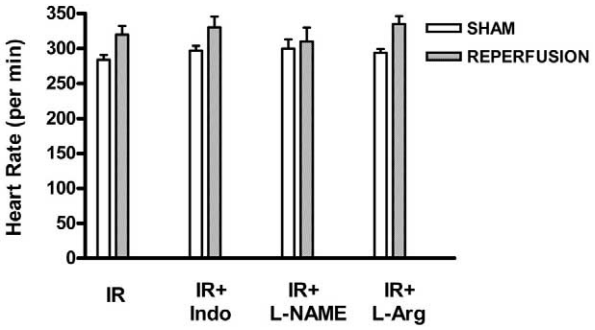

Fig 1. The effect of suprarenal aortic clamping and reperfusion on heart rate. The heart rate was continuously monitored at basal time period, during 30 minutes of suprarenal aortic clamping (ischemia) followed by 60 minutes of reperfusion and compared to a sham operation. Both groups were treated with either saline carrier (ischemia/reperfusion [*IR*] group, N = 10), indomethacin (*Indo*) (10 mg/kg [IR + Indo, N = 9], cyclooxygenase inhibitor), N^G-nitro-L-arginine methyl ester (*L-NAME*) (20 mg/kg, nitric oxide synthase inhibitor, N = 5), or L-arginine (*L-Arg*) (200 mg/kg, nitric oxide precursor, N = 7). Data are reported as beats per minute and expressed as mean ± SEM.

lecular weight standard markers (Sigma). Proteins were then transferred from the resolving gels to nitrocellulose membranes (Schleicher & Schuell, Keene, NH) as described by Burnette[33] using a Trans-Blot electrophoretic transfer cell (Bio-Rad) operating at 100 mA for 18 hours at 40°C. The membranes were incubated for 1 hour at room temperature in blocking buffer (buffer B: 10 mmol/L sodium phosphate, 0.5 mol/L NaCl, 10% bovine serum albumin, 0.05% Tween 20) and subsequently overnight at room temperature in buffer B containing a primary antibody,[19,34] α-COX-2 polyclonal antiserum (Cayman Chemical Co.), or α-iNOS antiserum (Oxford Biodmedical Research, Inc., Oxford, Mich). The nitrocellulose membranes were then washed of excess primary antibody at room temperature in phosphate-buffered saline Tween buffer (PBST: 10 mmol/L potassium phosphate, pH 7.4, 0.9% sodium chloride, 0.05% Tween 20) and incubated for 1 hour at room temperature in buffer B and secondary antibody, goat "-mouse IgG horseradish peroxidase (Bio-Rad). The membranes were washed of secondary antibody at room temperature in PBST, incubated in chemiluminescence reagent (Renaissance; DuPont-NEN, Boston, Mass) for 1 minute, inserted into a plastic sheet protector, bubbles removed, and placed against autoradiography film (Kodak, Rochester, NY). The film exposure times were adjusted to obtain the ideal density of bands identifying the enzymes of interest, and an imaging system (Alpha Innotech, San Leandro, Calif) was used to measure these bands by spot densitometry.

Creatinine clearance. Separate groups of animals were used to measure serum creatinine, urine creatinine, and urine volume. These groups underwent the same supra-SMA aortic clamping (and SH) followed by reperfusion as the above groups but without placement of microdialysis probes or laser Doppler fibers placed in the kidneys. These groups were subjected to the SRACR model with and without indomethacin, L-NAME or L-arginine as described above. Serum and urinary creatinine were measured by a SYNCHRON LC System (Beckman Coulter Inc., Fullerton, Calif).

Statistical analysis. Data are expressed as mean ± SEM. Analysis of variance and Student's *t* tests were used to determine differences among the groups. $P < .05$ value was considered statistically significant.

RESULTS

SRACR did not alter heart rate compared to the SH group. Treatment of the SRACR or SH groups with indomethacin, L-NAME of L-arginine did not alter heart rate as well (Fig 1). SRACR decreased blood pressure slightly compared to the SH group. As expected, L-NAME increased blood pressure and L-arginine decreased blood pressure compared to the baseline predrug measurements (Fig 2).

Suprarenal aortic clamping for 30 minutes markedly decreased both cortical and medullary blood flow. Following reperfusion for 60 minutes, cortical and medullary blood flow was still significantly decreased compared to basal levels, but the decrease was lessened by 50% compared to the 30-minute ischemia period (Figs 3 and 4). Treatment of the animals with either indomethacin or L-NAME during SRACR significantly decreased cortical and medullary blood flow following reperfusion compared to the ischemia/reperfusion group without drug treatment.

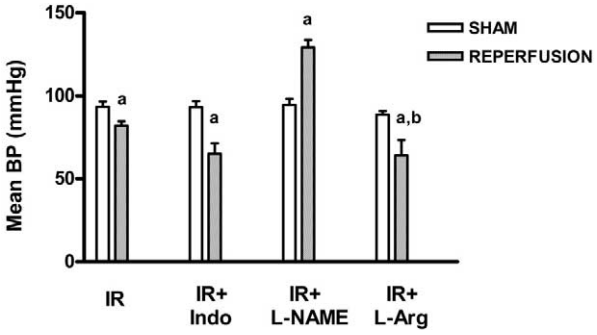

Fig 2. The effect of suprarenal aortic clamping and reperfusion on mean blood pressure. The mean blood pressure was continuously monitored at basal time period, during 30 minutes of suprarenal aortic clamping (ischemia) followed by 60 minutes of reperfusion and compared to a sham operation. Both groups were treated with either saline carrier (ischemia/reperfusion [*IR*] group, N = 10), indomethacin (*Indo*) (10 mg/kg [IR + Indo, N = 9], cyclooxygenase inhibitor), N^G-nitro-L-arginine methyl ester (*L-NAME*) (20 mg/kg, nitric oxide synthase inhibitor, N = 5), or L-arginine (*L-Arg*) (200 mg/kg, nitric oxide precursor, N = 7). Data are reported as mm Hg and expressed as mean ± SEM. *a* indicates significance at $P < .05$ compared to sham; *b* indicates significance at $P < .05$ compared to the IR group without drug treatment; *c* indicates significance at $P < .05$ compared to the L-NAME group. *BF*, blood flow.

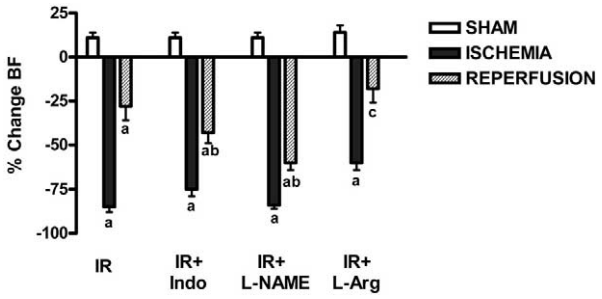

Fig 3. The effect of suprarenal aortic clamping and reperfusion on renal cortical blood flow. Laser Doppler probes were placed 2 mm into the cortex and blood flow (*BF*) was continuously monitored at basal time period, during 30 minutes of suprarenal aortic clamping (ischemia) followed by 60 minutes of reperfusion and compared to a sham operation. Both groups were treated with either saline carrier (ischemia/reperfusion [IR] group, N = 10), indomethacin (*Indo*) (10 mg/kg [IR + Indo, N = 9], cyclooxygenase inhibitor), N^G-nitro-L-arginine methyl ester (*L-NAME*) (20 mg/kg, nitric oxide synthase inhibitor, N = 5), or L-arginine (*L-Arg*) (200 mg/kg, nitric oxide precursor, N = 7). Data are reported as the percentage of change from basal time zero and expressed as mean ± SEM. *a* indicates significance at $P < .05$ compared to sham; *b* indicates significance at $P < .05$ compared to the IR group without drug treatment; *c* indicates significance at $P < .05$ compared to the L-NAME group.

Treatment of the animals with L-arginine prior to SRACR significantly lessened the decrease in cortical and medullary blood flow following reperfusion compared to the ischemia/reperfusion and L-NAME groups (Figs 3 and 4). In fact, L-arginine pretreatment almost completely reversed the decrease in medullary blood flow to basal (prior to placement of the aortic clamp) levels following reperfusion (Fig 4).

Suprarenal aortic clamping for 30 minutes led to a profound decrease in cortical and medullary NO release when compared to the SH level. Both cortical and medullary NO release was restored somewhat toward the SH level following 60 minutes of reperfusion (Figs 5 and 6). Treatment of the animals with L-NAME did not significantly alter the low cortical or medullary NO release that was present following SRACR in the ischemia/reperfusion group (treated with carrier only). L-Arginine pretreatment restored cortical NO release to the SH level following SRACR but did not restore medullary NO release to SH levels following SRACR (Figs 5 and 6).

Suprarenal aortic clamping for 30 minutes did not significantly alter cortical or medullary PGE_2 release following suprarenal aortic clamping or reperfusion when compared to the SH level. Both cortical and medullary PGE_2 release was significantly decreased to very low levels following SRACR and pretreatment with indomethacin (Figs 7 and 8).

SRACR did not alter iNOS content in the cortex but did induce a significant decrease in iNOS content in the medulla. SRACR did not alter COX-2 content in the cortex or medulla (Fig 9).

SRACR caused a marked decrease in the creatinine clearance compared to the SH group. Treatment with indomethacin, L-NAME, or L-arginine did not alter creatinine clearance following SRACR (Fig 10).

DISCUSSION

Over the past several decades considerable progress has been made in the management of patients undergoing suprarenal aortic reconstructive surgery. Despite these advances, renal dysfunction continues to be a frequent complication of suprarenal aortic reconstruction. Giulini et al[1] examined their results in 7534 consecutive patients treated with elective abdominal aortic aneurysm surgical repair from 1992 to 1999. Of this group, 56 patients had juxtarenal aneurysms that required suprarenal aortic clamping. The average time of renal exclusion was 20 minutes in the group treated with suprarenal aortic clamping. Renal function deteriorated in eight (14%) of the suprarenal group compared to none in group treated with infrarenal aortic clamping. Only one of the eight patients with renal deterioration required permanent dialysis.

Whalber et al[2] reviewed 60 consecutive patients between 1987 and 1994 who were treated for an abdominal aortic aneurysm or aortic occlusive disease that required temporary suprarenal aortic clamping during the aortic reconstruction. In this excellent series, none of the surviving patients required dialysis, but transient azotemia occurred in 23% of the patients. Odds ratios for the transient renal function showed a 10-fold increased risk of renal dysfunction when the suprarenal aortic

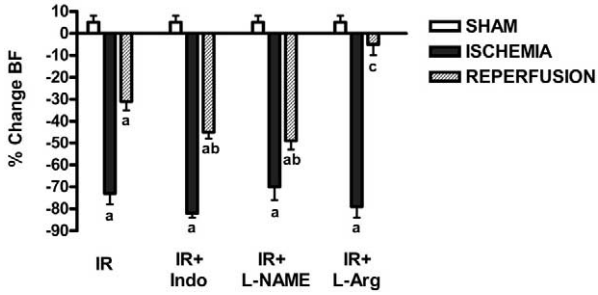

Fig 4. The effect of suprarenal aortic clamping and reperfusion on renal medullary blood flow. Laser Doppler probes were placed 4 mm into the medulla and blood flow (*BF*) was continuously monitored at basal time period, during 30 minutes of suprarenal aortic clamping (ischemia) followed by 60 minutes of reperfusion and compared to a sham operation. Both groups were treated with saline carrier (ischemia/reperfusion [*IR*] group, N = 10), indomethacin (*Indo*) (10 mg/kg [IR + Indo, N = 9], cyclooxygenase inhibitor), N^G-nitro-L-arginine methyl ester (*L-NAME*) (20 mg/kg, nitric oxide synthase inhibitor, N = 5), or L-arginine (*L-Arg*) (200 mg/kg, nitric oxide precursor, N = 7). Data are reported as the percentage of change from basal time zero and expressed as mean ± SEM. *a* indicates significance at $P < .05$ compared to sham; *b* indicates significance at $P < .05$ compared to the IR group without drug treatment; *c* indicates significance at $P < .05$ compared to the L-NAME group.

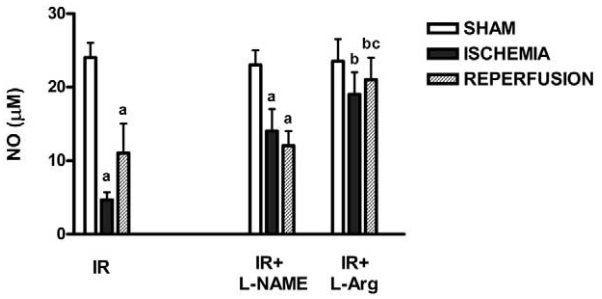

Fig 5. The effect of suprarenal aortic clamping and reperfusion on renal cortical nitric oxide (*NO*) release. Microdialysis probes were placed 2 mm into the cortex, connected to a syringe pump, and perfused in vivo at 3 μL/min with lactated Ringer solution. Dialysate fluid was collected at basal time zero, following 30 minutes of suprarenal aortic clamping (ischemia) followed by 60 minutes of reperfusion and compared to a sham operation. Both groups were treated with saline carrier (ischemia/reperfusion [*IR*] group, N = 10), indomethacin (*Indo*) (10 mg/kg [IR + Indo, N = 9], cyclooxygenase inhibitor), N^G-nitro-L-arginine methyl ester (*L-NAME*) (20 mg/kg, NO synthase inhibitor, N = 5), or L-arginine (*L-Arg*) (200 mg/kg, NO precursor, N = 7). Data are reported as μmol/L and expressed as mean ± SEM. *a* indicates significance at $P < .05$ compared to sham; *b* indicates significance at $P < .05$ compared to the IR group without drug treatment; *c* indicates significance at $P < .05$ compared to the L-NAME group.

clamp time was greater than 50 minutes.[2] It is clear, even in the best of surgical series, aortic reconstruction requiring suprarenal aortic clamping continues to be complicated by the potential for subsequent loss of renal function following SRACR.

The pathophysiology of renal azotemia following SRACR is not well understood. Very few experimental studies have examined the effect of SRACR on renal blood flow and function. Several animal models have shown that suprarenal aortic clamping decreases renal blood flow, GFR, urine flow, and fractional sodium reabsorption and an increase in renal vascular resistance following SRACR.[4-6] Mitaka et al[4] studied the effects of 1.5 hours of SRACR on canine renal function. The animals subjected to SRACR demonstrated a decrease in urine volume, renal blood flow, and creatinine clearance. These SRACR-induced decreases in renal function were reversed by prior treatment with atrial natriuretic peptide, suggesting that this protein may be helpful in protecting renal function following ischemia/reperfusion injury.[4] Another group of studies has shown that endothelin contributes to decreased renal blood flow, GFR, urine flow, fractional sodium, and an increase in renal vascular resistance following SRACR.[5,6] Unfortunately, none of these studies examined the effect of SRACR on the anatomic distribution and patterns of synthesis of endogenous renal vasodilators and the subsequent role of these changes on renal blood flow and function.

Endogenous renal prostanoids have been hypothesized to be potent mediators of renal vascular resistance for more than 30 years.[14-20] PGE_2 has been shown to be the major renal prostanoid synthesized by the kidney, although the kidney can also synthesize significant levels of PGI_2. Both of these arachidonic acid metabolites are potent vasodilators and the decreased synthesis of renal PGE_2 and PGI_2 has been associated with renal vasoconstriction.[15-20] The loss of endogenous renal vasodilators has been shown following a number of injuries including renal ischemia/reperfusion injury,[18-21] intestinal ischemia/reperfusion injury,[19,35,36] and acute burn.[37,38] In contrast to acute injury, the kidney responds to many types of chronic injuries (hydronephrosis, elevated renal venous pressure, ischemia) by altering endogenous eicosanoid prostanoid release to reflect a net increase in the synthesis of the potent vasoconstrictor thromboxane A_2.[16,17] In one example, 72 hours of hydronephrosis profoundly increased the endogenous synthesis and release of thromboxane in the renal cortex. The authors of this study hypothesized that the increased release of cortical thromboxane was involved with regulation of cortical blood flow during this injury. Thus, increased vasoconstrictor eicosanoid release can contribute to decreased renal blood flow following chronic renal injury.[16,17]

The continuous basal release of NO within the renal microvasculature has been shown to contribute to the maintenance of normal renal blood flow and function under normal physiologic conditions.[7-13] Loss of renal NO synthesis has been suggested to contribute to renal vasoconstriction following renal ischemia/reperfusion injury.[7-13,20,21,39] Renal ischemia in the rat increased renal vascular resistance, which could be reversed with

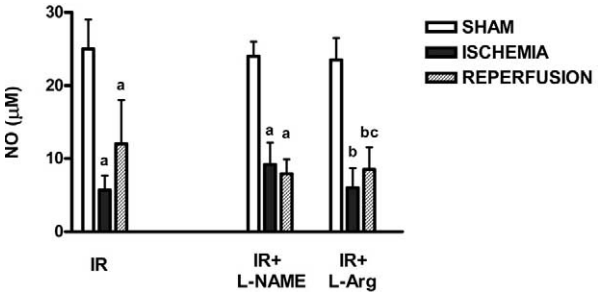

Fig 6. The effect of suprarenal aortic clamping and reperfusion on renal medullary nitric oxide (*NO*) release. Microdialysis probes were placed 4 mm into the medulla, connected to a syringe pump, and perfused in vivo at 3 μL/min with lactated Ringer solution. Dialysate fluid was collected at basal time zero, following 30 minutes of suprarenal aortic clamping (ischemia) followed by 60 minutes of reperfusion and compared to a sham operation. Both groups were treated with saline carrier (ischemia/reperfusion [*IR*] group, N = 10), indomethacin (*Indo*) (10 mg/kg [IR + Indo, N = 9], cyclooxygenase inhibitor), N^G-nitro-L-arginine methyl ester (*L-NAME*) (20 mg/kg, nitric oxide synthase inhibitor, N = 5), or L-arginine (*L-Arg*) (200 mg/kg, NO precursor, N = 7). Data are reported as μmol/L and expressed as mean ± SEM. *a* indicates significance at $P < .05$ compared to sham; *b* indicates significance at $P < .05$ compared to the IR group without drug treatment; *c* indicates significance at $P < .05$ compared to the L-NAME group.

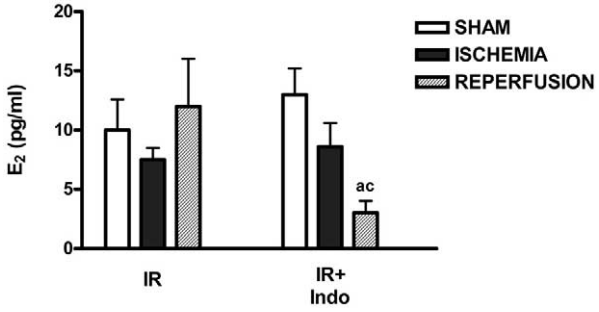

Fig 7. The effect of suprarenal aortic clamping and reperfusion on renal cortical prostaglandin E_2 (*E_2*) release. Microdialysis probes were placed 2 mm into the cortex, connected to a syringe pump, and in vivo at 3 μL/min with lactated Ringer solution. Dialysate fluid was collected at basal time zero, following 30 minutes of suprarenal aortic clamping (ischemia) followed by 60 minutes of reperfusion and compared to a sham operation. Both groups were treated with saline carrier (ischemia/reperfusion [*IR*] group, N = 10), indomethacin (*Indo*) (10 mg/kg [IR + Indo, N = 9], cyclooxygenase inhibitor), N^G-nitro-L-arginine methyl ester (*L-NAME*) (20 mg/kg, nitric oxide synthase inhibitor, N = 5), or L-arginine (*L-Arg*) (200 mg/kg, nitric oxide precursor, N = 7). Data are reported as pg/mL PGE_2 release and is expressed as mean ± SEM. *a* indicates significance at $P < .05$ compared to sham; *b* indicates significance at $P < .05$ compared to the IR group without drug treatment; *c* indicates significance at $P < .05$ compared to the L-NAME group.

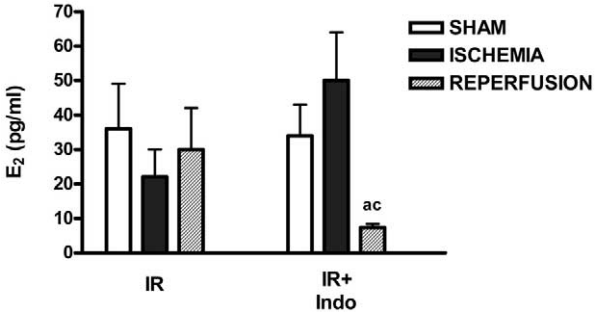

Fig 8. The effect of suprarenal aortic clamping and reperfusion on renal medullary prostaglandin E_2 (*E_2*) release. Microdialysis probes were placed 2 mm into the cortex, connected to a syringe pump, and in vivo at 3 μL/min with lactated Ringer solution. Dialysate fluid was collected at basal time zero, following 30 minutes of suprarenal aortic clamping (ischemia) followed by 60 minutes of reperfusion and compared to a sham operation. Both groups were treated with saline carrier (ischemia/reperfusion [*IR*] group, N = 10), indomethacin (*Indo*) (10 mg/kg [IR + Indo, N = 9], cyclooxygenase inhibitor), N^G-nitro-L-arginine methyl ester (*L-NAME*) (20 mg/kg, nitric oxide synthase inhibitor, N = 5), or L-arginine (*L-Arg*) (200 mg/kg, nitric oxide precursor, N = 7). Data are reported as pg/mL PGE_2 release and expressed as mean ± SEM. *a* indicates significance at $P < .05$ compared to sham; *b* indicates significance at $P < .05$ compared to the IR group without drug treatment; *c* indicates significance at $P < .05$ compared to the L-NAME group.

infusion of L-arginine but not with NOS inhibition.[7] Depletion of renal arginine led to was show to decrease renal perfusion rate, GFR, and urine flow rate, which could be completely reversed with L-arginine (not D-arginine) repletion. These results suggested that L-arginine was an important amino acid in the regulation of renal NO synthesis and renal function.[10] A more recent study used micropuncture techniques in the rat to examine the role of NO in glomerular arteriolar microvascular blood flow and renal function. The results showed that intravenous L-N^G-monomethyl-L-arginine (an NOS inhibitor) caused glomerular arteriolar vasoconstriction (with a predominant effect on the efferent arteriole), decreased ultrafiltration, and increased glomerular capillary pressure.[11,12]

A study quite relevant to the present study compared the effect of NOS inhibition on renal function in the in vitro and in vivo perfused rat kidney models. In the experiments using the in vitro perfused rat kidney, NOS inhibition caused an increase in renal vascular resistance that was reversed by L-arginine infusion. In the experiments using the in vivo kidney, NOS inhibition increased systemic arterial pressure, decreased GFR, and decreased renal plasma flow.[13] Zou and Cowley[39] used an in vivo microdialysis technique to determine regional differences of renal NO production. These authors found that there was a higher NO concentration in the medulla than the cortex. The authors concluded that NO may play a role in the control of vascular tone and tubular function in the renal medulla. Although these studies suggest that NO is an important endogenous renal vasodilator in the maintenance of renal blood flow, the effect of SRACR on renal cortical and medullary NO synthesis has remained undefined.

Several studies have examined the hypothesis that renal ischemia decreases renal blood flow and endoge-

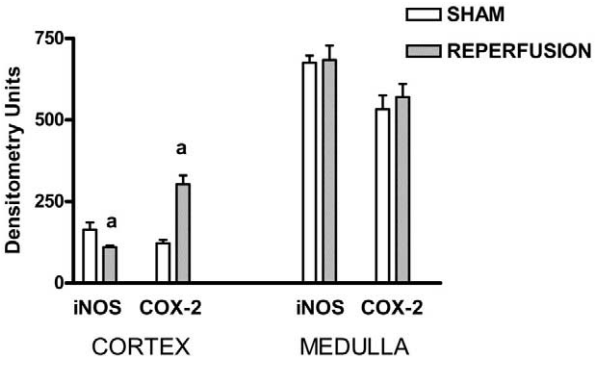

Fig 9. The effect of suprarenal aortic clamping and reperfusion on renal cortical and medullary inducible nitric oxide synthase (*iNOS*) and cyclooxygenase 2 (*COX-2*) content. Protein from renal cortex and medulla was prepared from sham and suprarenal aortic clamping and reperfusion animals and subjected to Western blot analysis to identify content of iNOS and COX-2. The blots were analyzed by densitometry. Data are expressed as densitometry units ± SEM (N = 8). *a* indicates significance at $P < .05$ compared to sham.

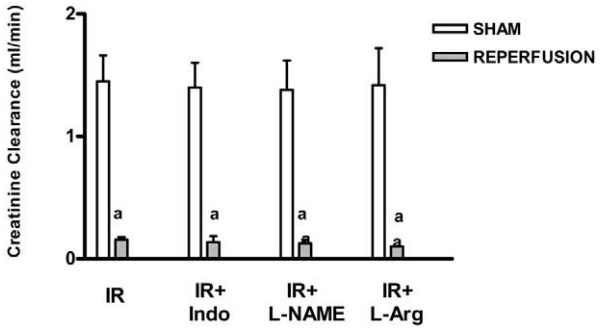

Fig 10. The effect of suprarenal aortic clamping and reperfusion on creatinine clearance. Serum and urine were collected from separate groups of animals to measure serum creatinine, urine creatinine, and urine volume to calculate creatinine clearance. The serum and urine were collected at the time zero of each experiment and at the end of reperfusion. These groups underwent the same above the superior mesenteric artery aortic clamping (and sham) followed by reperfusion without drug treatment (ischemia/reperfusion [*IR*], N = 8), treatment with indomethacin (*Indo*) (IR + Indo, N = 5), treatment with N^G-nitro-L-arginine methyl ester (*L-NAME*) (IR + L-NAME, N = 6), and treatment with L-arginine (*L-Arg*) (IR + L-Arg, N = 5). Data are reported as mL/min and expressed as mean ± SEM. *a* indicates significance at $P < .05$ compared to sham.

nous renal vasodilator eicosanoids and NO. The first study showed that inhibition of either NO synthesis or COX enhanced the increase in renal vascular resistance following renal ischemia and reperfusion. The authors concluded that vasodilator eicosanoids and NO are important mediators involved with maintenance of renal blood flow after ischemia/reperfusion injury.[20] A second group of studies suggest that acute renal injury induces changes in blood flow away from the deep cortex and outer medulla.[16,17,22-26] There is no literature regarding the effects of SRACR on regional blood flow in the kidney.

The current study is the first to use both in vivo microdialysis and microlaser Doppler fibers to investigate in vivo real-time changes in renal cortical and medullary blood flow, NO, and PGE_2 synthesis following SRACR. In the present study, temporary occlusion of the abdominal aorta above the SMA caused a marked decrease in medullary and cortical blood flow with a concomitant decrease in endogenous medullary and cortical NO synthesis. Treatment with L-NAME (NOS inhibitor) further decreased blood flow and NO synthesis in the medulla and cortex. L-Arginine restored medullary and cortical NO synthesis and blood flow in the cortex (but not the medulla). SRACR did not alter renal medullary or cortical release of PGE_2. However, the addition of indomethacin, a COX inhibitor, caused a concomitant decrease in medullary and cortical PGE_2 synthesis and blood flow. The Western blot data suggest that the loss of endogenous cortical NO could contribute to the decreased cortical blood flow found following the SRACR model and may be one of the mechanisms that contribute to increased cortical vascular resistance following renal injury. The creatinine clearance data suggest that even our model of short-term SRACR induced a profound decrease in renal function that was not reversed by treatment with the NO precursor L-arginine. This finding suggests that there are other factors that contribute to decreased renal function following SRACR and that our future studies should determine the earliest time period of SRACR in which reversing the decrease in renal cortical and medullary blood flow will still maintain normal renal function.

In summary, the findings of this study suggest that NO is an important endogenous renal vasodilator that, when maintained, can help preserve cortical blood flow following SRACR. The data in Figures 6 and 9 suggest that the downregulation of in vivo microdialysis renal medullary NO release following SRACR was due in part to a decrease in cortical iNOS content. Interestingly, these data also suggest that avoidance of COX inhibitors can help maintain endogenous renal cortical and medullary PGE_2 synthesis and thus contribute to maintaining normal blood flow following SRACR injury. Identification of factors responsible for maintaining renal microvascular blood flow will allow development of treatment strategies that will help maintain renal microvascular blood flow and function in the treatment of complex aortic pathology that requires suprarenal aortic clamping during the aortic reconstruction.

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