N-acetyl-L-cysteine improves renal medullary hypoperfusion in acute renal failure

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Conesa, Erica López, Fernandez Valero, Jose Carlos Nadal, Francisco J. Fenoy, Bernardo Lopez, Begona Arregui, and Miguel Garcia Salom. N-acetyl-L-cysteine improves renal medullary hypoperfusion in acute renal failure. Am J Physiol Regulatory Integrative Comp Physiol 281: R730–R737, 2001.—This study evaluated the effects of N-acetyl-L-cysteine (NAC), a free radical scavenger, and N-nitro-l-arginine methyl ester (l-NAME), a nitric oxide (NO) synthesis inhibitor, on the changes in renal function, intrarenal blood flow distribution (laser-Doppler flowmetry), and plasma peroxynitrite levels during the acute renal failure (ARF) produced by inferior vena cava occlusion (IVCO; 45 min) in anesthetized rats. Renal blood flow fell on reperfusion (whole kidney by −45.7%; cortex −58.7%, outer medulla −62.8%, and papilla −47.7%); glomerular filtration rate (GFR) also decreased (−68.6%), whereas fractional sodium excretion (FE\textsubscript{\text{Na\textsubscript{x}}}) and peroxynitrite and NO\textsubscript{2}/NO\textsubscript{3} plasma levels increased (189.5, 46.5, and 390%, respectively) after ischemia. Pretreatment with l-NAME (10 mg·kg\textsuperscript{-1}·min\textsuperscript{-1}) aggravated the fall in renal blood flow seen during reperfusion (−60%). Pretreatment with NAC (150 mg·kg bolus + 715 mg·kg\textsuperscript{-1}·min\textsuperscript{-1}) partially prevented those changes in renal function (GFR only fell by −29.2%, and FE\textsubscript{\text{Na\textsubscript{x}}} increased 119.4%) and laser-Doppler blood flow, especially in the outer medulla, where blood flow recovered to near control levels during reperfusion. These beneficial effects seen in rats given NAC seem to be dependent on the presence of NO, because they were abolished in rats pretreated with l-NAME. Also, the antioxidant effects of NAC prevented the increase in plasma peroxynitrite after ischemia. In conclusion, NAC ameliorates the renal failure and the outer medullary vasoconstriction induced by IVCO, effects that seem to be dependent on the presence of NO and the scavenging of peroxynitrite.

inferior vena cava occlusion; free radical scavengers; peroxynitrite; nitric oxide; plasma nitrite/nitrate; renal function; laser-Doppler flowmetry

RENAL ISCHEMIA IS OBSERVED in a variety of clinical situations such as cardiac arrest with recovery, liver transplantation, heminephrectomy, etc. The acute renal failure (ARF) observed after ischemia is characterized by decreased glomerular filtration rate (GFR), tubular necrosis, and increased renal vascular resistance (RVR; 2, 17, 27). The pathophysiological changes responsible for the postischemic renal injury and the profoundly depressed renal function remain incompletely understood. It has been suggested that abnormalities in the renal circulation persist in the postischemic period after the reflow and contribute to the impaired renal function (2, 11, 17, 20).

Although total renal blood flow (RBF) is reduced in postischemic ARF (30–50% below normal values), this hemodynamic alteration seems to be insufficient to account for the profound fall in GFR that characterizes ARF. However, because the renal medulla is normally on the verge of hypoxia, the renal vasoconstriction observed during reperfusion could play an important role in the development of ARF, principally by determining a state of prolonged hypoperfusion within the outer medulla, where a high oxygen consumption is needed for active solute transport. This medullary alteration seems to be related to endothelial dysfunction, leukocyte activation, and leukocyte-endothelial adhesion (12, 17, 25). There is indirect evidence showing that endothelial dysfunction appears to be due to the generation of oxygen free radicals during reperfusion (17, 25, 28). It is known that when infused before reperfusion, oxygen free radical scavengers exert a beneficial effect by preventing oxygen free radical production during reoxygenation (1, 10, 22, 25, 28). In addition, the beneficial effect of some scavengers has been attributed to nitric oxide (NO) potentiation (3, 19, 25), suggesting that the inactivation of NO is an important factor contributing to postischemic ARF. Thus it can be hypothesized that the beneficial effects of free radical scavenging on renal function may be exerted by protecting NO from inactivation, thus improving outer medullary vascular congestion during reperfusion. However, at present, the effects of free radical scavengers on the postischemic changes in the renal circulations are unknown.

Therefore, the aim of the present study was to investigate the effect of N-acetyl-l-cysteine (NAC), a free radical scavenger, on renal function during postischemic reperfusion. Briefly, rats were subjected to ischemia and reperfusion and then were divided into groups that received NAC (150 mg·kg bolus + 715 mg·kg\textsuperscript{-1}·min\textsuperscript{-1}) or saline (s) immediately after reperfusion. Pretreatment with NAC partially prevented the decrease in RBF seen during reperfusion (−60%). Pretreatment with NAC decreased plasma peroxynitrite levels (−35.4%, −27.3%, and −36%, respectively) and increased GFR (2, 11, 17, 20).

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radical scavenger, on the intrarenal blood flow changes observed in an experimental model of ischemic ARF, induced by inferior vena cava occlusion above the renal veins, thus blocking renal venous drainage with subsequent renal hypoperfusion. This maneuver is similar to that observed during the anhepatic phase of the orthotopic liver transplant, when inferior cava vein is blocked above renal veins. Recently, it was reported that this model of ischemic ARF causes renal endothelial dysfunction and renal vasoconstriction that are partially prevented by pretreatment with NAC (25), a free radical scavenger able to enhance the biological effects of NO by forming an S-nitrosothiol (15), thus suggesting that NO is involved in the beneficial effect of NAC in this model of ischemia-reperfusion. Consequently, in the present study, the possible role of NO in mediating the effects of NAC on the postischemic changes in the intrarenal distribution of blood flow was also evaluated.

METHODS

Experiments were performed on 89 Munich-Wistar rats (200–250 g body wt) purchased from Harlan Laboratories (Madison, WI) and bred in our animal care facility. All procedures were in accordance with the recommendations of the Declaration of Helsinki and the guiding principles in the care and use of animals approved by the Council of the American Physiological Society. Rats were anesthetized with an injection of ketamine (30 mg/kg im) and Inactin (50 mg/kg ip) and placed on a heated table to maintain body temperature at 36.5°C. Cannulas were placed in the jugular vein for infusions and in the femoral artery for arterial pressure measurements. The infrahepatic inferior cava vein was isolated, and a tie was loosely placed around it to allow for cava vein occlusion. Rats received an intravenous infusion of a 0.9% sodium chloride solution containing 1% bovine serum albumin at a rate of 2 ml·100 g⁻¹·h⁻¹ throughout the experiment. After completion of the surgical procedure, [³H]inulin (1 μCi/ml) was added to the intravenous infusion, and a 60- to 90-min equilibration period was allowed before the experimental protocol was accomplished.

Renal function experiments. These rats were surgically prepared as described above. In addition, a noncannulating electromagnetic flow probe (Skalar Medical, model 1401) was placed around the renal artery for RBF measurements, and the ureter was cannulated for urine sampling. Midpoint plasma samples were drawn from the femoral artery to allow for measurement of GFR. RVR was calculated from renal perfusion pressure and RBF values. In these experiments, urine flow, sodium excretion, RBF, GFR, and arterial pressure were measured during a 30-min control period. Then either vehicle (group 1, n = 8), NAC (group 2, 150 mg/kg as a bolus plus 715 μg·kg⁻¹·min⁻¹, n = 7), L-NAME (group 3, 10 μg·kg⁻¹·min⁻¹, n = 6), or L-NAME plus NAC (group 4, same doses as in groups 2 and 3, n = 6) were administered intravenously (until the end of the experiment), and, after a 30-min equilibration period, urine and plasma samples were collected again in a 30-min experimental clearance period. A hemostatic clamp was then tightened around the inferior vena cava above the renal veins for 45 min, and plasma and urine samples were collected again. After the 45-min period, the clamp was removed, enabling renal reperfusion, and four consecutive 30-min clearance periods were performed.

The doses of NAC and L-NAME used in the present study are similar to those used in previous experiments in dogs (25), in which NAC ameliorated the renal failure and renal endothelial dysfunction induced by inferior vena cava occlusion. In a previous study, this dose of L-NAME (10 μg·kg⁻¹·min⁻¹) blunted pressure natriuresis and diuresis and decreased papillary blood flow at high renal perfusion pressure (6).

Laser-Doppler blood flow experiments. The left kidney was placed dorsally side up in a holder positioned above the abdominal aorta. The papilla was exposed by making a longitudinal incision in the ureter from the tip to the base of the papilla. Papillary blood flow (PBF; arbitrary units) was measured by laser-Doppler flowmetry (dual-channel laser blood flow monitor, model MBF3D, Moor Instruments) by placing a fiberoptic probe (P4, 1.0 mm OD) 1 mm from the tip of the papilla. Outer medullary blood flow (OMBF; arbitrary units) was measured by introducing a needle probe (P4s, 0.5 mm OD) through the renal cortex 2.5 mm in depth. The location of the probe tip in the outer medulla was verified in all animals by dissecting the kidney at the end of the experiment. The needle probe was secured in a stable position by using a micromanipulator (Prior). The depth of penetration was marked in the probe using a waterproof pen, and the movements of the kidney during the experiment were followed under the microscope by continuously adjusting the position of the probe using the manipulator. Cortical blood flow (CBF; arbitrary units) was measured by placing the probe (P4, 1 mm OD) at three random locations on the dorsal surface of the kidney; the mean flow signal from these areas is reported. The laser-Doppler flowmeter was calibrated by using a colloid suspension of latex particles; the Brownian motion of these particles (at standard temperature, 22°C) was used as a “motility standard.” The probes were introduced into the suspension, and the gain of the instrument was adjusted to obtain a flow signal of 250 U (±5%). The same calibration was used for papillary, outer medulla, and cortical measurements.

After surgery and a 1-h equilibration period, PBF, OMBF, and CBF were measured under control conditions and re-determined 30 min after the administration of either vehicle (control, group 10, n = 6), NAC (group 11, 150 mg/kg as a bolus plus 715 μg·kg⁻¹·min⁻¹, n = 6), L-NAME (group 12, 10 μg·kg⁻¹·min⁻¹, n = 6), or L-NAME plus NAC (group 13, same doses as in groups 11 and 12, n = 6). Ocluding inferior vena cava above renal veins for 45 min then induced ischemia. PBF, OMBF, and CBF were monitored during this 45-min ischemic period. After that, the clamp was released and OMBF and CBF were measured over four consecutive 30-min periods during reperfusion.

In vivo oxidation of dihydrorhodamine 123 (DHR) to rhodamine 123 as an index of peroxynitrite production. In five different groups of animals (following the same experimental protocols), plasma concentration of peroxynitrite was determined as described (21, 23, 26). In the time control group (group 9), plasma samples were drawn in basal conditions, with no ischemia. In the remaining four groups, the rats were surgically prepared as described above, and 60 min after surgery, either saline (group 10), NAC (group 11), L-NAME (group 12), or L-NAME plus NAC (group 13) were administered until the end of the experiment. Thirty minutes later, ARF was induced by clamping the inferior vena cava vein for 45 min. Twenty-five minutes after reperfusion, DHR (2 μmol/kg in 0.5 ml of saline) was infused intravenously and, 20 min later, blood samples were collected into heparinized tubes for rhodamine 123 fluorescence determination. It was previously reported that the amount of peroxynitrite formed is maximal

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45 min after reperfusion (26). In the time control group, DHR was given 100 min after surgical preparation. Blood samples were centrifuged (5,000 rpm, 5 min, 4°C), and plasma was stored at −80°C in separate tubes until determination of rhodamine 123 by fluorescence spectroscopy.

The oxidation of DHR to rhodamine 123 has been used to estimate the formation of ROS in vivo, as a way to indirectly quantify the peroxynitrite generated in vivo (21, 23, 26). Rhodamine 123 fluorescence was measured on a Hitachi fluorometer (excitation 500 nm, emission 533 nm, slit widths 2.5 nm) using quartz cuvettes containing 0.5 ml of plasma plus 0.5 ml of water. The results are expressed as the concentration (nM) of rhodamine 123 formed in vivo.

To demonstrate that DHR (2 μmol/kg) was administered in excess to measure all the peroxynitrite generated in vivo, plasma samples obtained from rats given DHR were incubated with 44 μg of horseradish peroxidase and 250 μM hydrogen peroxide for 1 h at 37°C. The maximal concentration of rhodamine 123 formed with this oxidant procedure was 13.49 nM, well above the levels measured in the ischemic groups in vivo, indicating that under our experimental conditions, the dose of DHR was enough to measure peroxynitrite levels in vivo.

The amount of rhodamine 123 formed in vivo was quantified by using a rhodamine standard curve (1–30 nM), prepared by using plasma obtained from untreated rats.

**Measurements of plasma NO$_2$ /NO$_3$ concentration.** Plasma NO$_2$ /NO$_3$ concentration, a marker of NO generation, was also measured as previously described in groups 9–13 in the same blood samples drawn to measure rhodamine 123. Briefly, plasma was deproteinized, and plasma nitrate was reduced to nitrite by incubation with nitrate reductase (670 μM/mL) and NADPH (160 μM) at room temperature. After 1 h of incubation, nitrite concentration in plasma samples was measured by the Griess reaction, by adding 100 μl of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine in 5% phosphoric acid) to 100 μl of plasma. The optical density at 550 nm (OD$_{550}$) was measured using a Microplate Reader 2001 (Bio Whittaker). Nitrate concentrations were calculated by comparison with OD$_{550}$ of standard solutions of potassium nitrate prepared in distilled water.

To exclude interferences between the Griess reaction and DHR, a separate group of rats (n = 4) were not given DHR, underwent the same protocol as group 10 animals (ischemic rats infused with saline), and plasma samples were drawn for NO$_2$ /NO$_3$ determination. No differences were observed in plasma NO$_2$ /NO$_3$ values between these animals and group 10 rats (58.30 ± 3.22 and 53.11 ± 8.25 μmol/l, respectively).

**Drugs.** Ketamine was obtained from Rhône-Mérieux, Inactin from Sigma-RBI, DHR and rhodamine 123 were obtained from Fluka (Sigma Chemical), and L-NAME was from Sigma-RBI.

Analytic methods. Urine volume was measured gravimetrically. [3H]inulin concentrations in urine and plasma samples were determined by liquid scintillation spectrophotometry. GFR was calculated as the ratio of urine to plasma inulin concentration times urine flow rate. The sodium concentration of urine and plasma samples was determined by flame photometry. RBF, GFR, urine, and Na$^+$ excretion were factored by gram kidney weight. FE$_{Na}$ indicates the percentage of sodium filtered that is excreted (FE$_{Na}$ = ([urine Na$^+$]/[plasma Na$^+$]⋅GFR)⋅100). RVR was calculated from RPP and RBF values, as RVR = RPP/RBF (mmHg⋅min⋅ml$^{-1}$⋅g$^{-1}$ of kidney weight).

**Statistical methods.** Data are presented as means ± SE. The significance of differences in the measured values between groups was analyzed using a two-way ANOVA for repeated measurements followed by a Fisher’s protected t-test. The significance in the measured values within groups was analyzed with a one-way ANOVA for repeated measures, followed by a Fisher’s protected t-test. A value of P < 0.05 (2-tailed test) was considered statistically significant.

**RESULTS**

Effects of NAC and L-NAME on renal function before ischemia. The effects of NAC, L-NAME, and L-NAME+NAC on renal function and RPP before ischemia are presented in Figs. 1–4. Because the changes observed in RPP were similar in the rats used to evaluate renal function and those used in the laser-Doppler flowmetry experiments, the data obtained in both studies from rats with the same treatment have been pooled and are presented in Fig. 1. No significant differences were observed in hemodynamic and excretory parameters among the experimental groups during the basal period. The administration of NAC (group 2) had no effects on renal function or RPP. The infusion of L-NAME in group 3 rats induced a significant increase in RPP from 141 ± 4 to 154 ± 6 mmHg (Fig. 1), a decrease in RBF from 8.2 ± 0.4 to 6.1 ± 0.4 ml/min⋅g$^{-1}$ (Fig. 2), and an increase in RVR from 17.6 ± 2.3 to 25.3 ± 2.4 mmHg⋅min⋅ml$^{-1}$⋅g$^{-1}$ (Fig. 3). However, GFR (Fig. 2) and FE$_{Na}$ (Fig. 4) did not change after L-NAME. Similar changes were observed when L-NAME was administered simultaneously with NAC (group 4).

Hematocrit was stable and similar in all experimental groups during preischemia (44.6 ± 1.1, 41.2 ± 1.0, calculated from RPP and RBF values, as RVR = RPP/RBF (mmHg⋅min⋅ml$^{-1}$⋅g$^{-1}$ of kidney weight).

**Fig. 1. Renal perfusion pressure in control conditions (Basal) and after the administration of saline (Control, n = 14), N-acetyl-L-cysteine (NAC; 150 mg/kg, as a bolus, plus 715 μg⋅kg$^{-1}$⋅min$^{-1}$, n = 13), N-nitro-L-arginine methyl ester (NAME; 10 μg⋅kg$^{-1}$⋅min$^{-1}$, n = 12), or NAME plus NAC (same doses as in groups NAC and NAME, n = 12), before (Drugs), during (Isch), and after ischemia (R1–R4; experimental periods starting at 0, 30, 60, and 90 min, respectively, after reperfusion). *Significant difference from the basal period of the same group. †Significant difference from the same experimental period of the control group.**
Renal function during ischemia. When the clamp was tightened around the inferior vena cava for 45 min, RPP fell significantly in all experimental groups (Fig. 1). However, in rats given L-NAME (group 3), the hypotension observed was lower than in the other groups. Also, RBF, GFR, and sodium and water excretion fell to near zero values in all groups during inferior vena cava occlusion (ICVO; Figs. 2 and 4), indicating ARF.

Renal function during reperfusion. In all groups, RPP recovered near preischemic values at the onset of reperfusion. In rats given L-NAME (groups 3 and 4), RPP was significantly higher than in control animals after ICVO (Fig. 1). RBF recovered partially at the onset of reperfusion (Fig. 2) in all groups and then decreased slowly along reperfusion, indicating a significant and progressive increase in RVR (Fig. 3). However, in NAC-pretreated rats (group 2), RBF and RVR recovered on reperfusion up to control levels and did not change afterward (Fig. 2). The recovery of GFR after reperfusion was similar in groups 1, 3, and 4 (31.4, 34.3, and 37.4%, respectively, of preischemic values after 120 min of reperfusion). In contrast, in NAC-pretreated rats, GFR reached 70.5% of the preischemic value 120 min after reperfusion.

\[ \text{FENa} \% = \frac{\text{Fractional excretion of sodium}}{100} \]

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Fig. 2. Renal blood flow (A) and glomerular filtration rate (B) in control conditions (Basal) and after the administration of saline (Control, \(n = 8\)), NAC (150 mg/kg, as a bolus, plus 715 μg·kg\(^{-1}\)·min\(^{-1}\), \(n = 6\)), NAME (10 μg·kg\(^{-1}\)·min\(^{-1}\), \(n = 6\)), or NAME plus NAC (same doses as in groups NAC and NAME, \(n = 6\)), before (Drugs), during (Isch), and after ischemia. *Significant difference from basal period of the same group. †Significant difference from the same experimental period of the control group.

Fig. 3. Renal vascular resistance (RVR) in control conditions (Basal) and after the administration of saline (Control, \(n = 8\)), NAC (150 mg/kg, as a bolus, plus 715 μg·kg\(^{-1}\)·min\(^{-1}\), \(n = 7\)), NAME (10 μg·kg\(^{-1}\)·min\(^{-1}\), \(n = 6\)), or NAME plus NAC (same doses as in groups NAC and NAME, \(n = 6\)), before (Drugs) and after ischemia. *Significant difference from the same experimental period of the control group.

Fig. 4. Fractional excretion of sodium (\(\text{FENa}\%\)) in control conditions (Basal) and after the administration of saline (Control, \(n = 8\)), NAC (150 mg/kg, as a bolus, plus 715 μg·kg\(^{-1}\)·min\(^{-1}\), \(n = 6\)), NAME (10 μg·kg\(^{-1}\)·min\(^{-1}\), \(n = 6\)), or NAME plus NAC (same doses as in groups NAC and NAME, \(n = 6\)), before (Drugs), during (Isch), and after ischemia. *Significant difference from the same experimental period of the control group.
Effects of NAC and L-NAME on the intrarenal distribution of blood flow (laser-Doppler flowmetry). No significant differences were observed in hemodynamic parameters among experimental groups during the basal period (Table 1). The administration of NAC (group 6) had no effects on the intrarenal distribution of blood flow. The infusion of L-NAME induced a slight, but significant, decrease in CBF in group 7 and OMBF in group 8 (Fig. 5).

Intrarenal blood flow changes during ischemia. CBF, OMBF, and papillary blood flow (PBF) fell in all groups (Fig. 5) near zero values, demonstrating that ICVO induced renal ischemia.

Intrarenal distribution of blood flow during reperfusion. CBF, OMBF, and PBF recovered partially at the onset of reperfusion (Fig. 5) in all groups. In addition, CBF in groups 5, 7, and 8 (control, L-NAME, and L-NAME+NAC) decreased significantly during reperfusion, in accordance with the progressive increase in whole kidney RVR observed in the renal function study (Fig. 3). In contrast, in NAC-pretreated rats, CBF recovered up to 71.2% of preischemic value and remained unaltered during reperfusion, so that 30 min after reperfusion, CBF was significantly higher than in control rats.

Similarly, OMBF and PBF fell during reperfusion in the control group (Fig. 5), indicating a progressive increase in vascular resistance in the renal medulla. In contrast, in NAC-pretreated animals, OMBF and PBF recovered after reperfusion to near preischemic values. This beneficial effect was more prominent in the outer medulla of rats given NAC, where blood flow fully recovered to basal values after reperfusion, indicating a protective effect of NAC on the renal medullary postischemic vasoconstriction. On the other hand, in L-NAME- and L-NAME+NAC-treated animals, OMBF and PBF values were similar to those observed in control group during reperfusion.

Peroxynitrite plasma concentration during reperfusion. The effects of ischemia-reperfusion on peroxynitrite plasma concentration are presented in Fig. 6A. Ischemia (group 10) elevated plasma peroxynitrite (46%, P < 0.01) compared with time control rats (group 9). A further increase in peroxynitrite (91.8%) was observed in ischemic animals given L-NAME (group 12). The infusion of NAC before ischemia prevented the increase of peroxynitrite observed in ischemic animals (group 11) and reduced the increase in peroxynitrite (52.3%) observed in ischemic rats given L-NAME (L-NAME+NAC, group 13).

<table>
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<th>Group</th>
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<tr>
<td>NAME+NAC</td>
<td>316.7 ± 9.7</td>
<td>83.7 ± 6.4</td>
<td>217.1 ± 6.6</td>
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Control (n = 6) rats infused with saline. NAC (n = 6), rats infused with N-acetyl-L-cysteine (150 mg/kg as a bolus plus 715 μg·kg⁻¹·min⁻¹). NAME (n = 6), rats infused with Nω-nitro-l-arginine methyl ester (10 μg·kg⁻¹·min⁻¹). NAME plus NAC (n = 6), rats infused with NAME plus NAC.
Ischemia-reperfusion is produced when the arterial supply to the kidney is mechanically interrupted. However, this situation can also be observed when renal venous drainage is blocked by inferior vena cava occlusion above renal veins, as occurs during the anhepatic phase of orthotopic liver transplantation (4, 29). Recently, this experimental model has been characterized in anesthetized dogs (25), demonstrating that ischemic ARF and endothelial dysfunction develop after IVCO above the renal veins. In the present study, during IVCO, RBF decreased to near zero values, accompanied by a significant fall in RPP. The renal ischemia was maintained for 45 min, inducing an ARF characterized by a profound fall in GFR and sodium and water excretion, associated with increased fractional sodium excretion. It has been suggested that this type of ARF is a consequence of the renal hypoperfusion due to the increase in renal venous pressure (5). In addition, renal venous congestion may have compressed renal tubules, thereby increasing tubular hydrostatic pressure near stop-flow pressure and contributing to the fall in GFR (7). Our results support this interpretation, because RBF was virtually abolished in control rats during IVCO, indicating that renal venous pressure increased, matching the levels of RPP.

When the clamp was released, RPP returned close to preischemic levels, but RBF and GFR recovered only partially in all groups. In control rats, RBF and GFR remained reduced during reperfusion (77.1 and 23.4%, respectively, of the preischemic values) in the presence of an elevated FENa, indicative of tubular dysfunction. During reperfusion, RBF decreased progressively in untreated rats (from 5.4 ± 0.6 on reflow to 3.8 ± 0.6 ml·min⁻¹·g⁻¹ 120 min after reperfusion), indicative of growing renal reperfusion damage. GFR did not change, and FENa decreased progressively along with the fall in GFR, although it remained significantly elevated 2 h after reflow. Pretreatment with NAC improved the ARF observed during reperfusion. RBF and GFR were significantly higher, and FENa was lower in rats given NAC than in control animals. This beneficial effect of NAC was abolished when NAC was simultaneously infused with L-NAME. A similar effect of NAC on RBF and GFR was previously reported after IVCO in dogs (25). However, in the same study, pretreatment with L-NAME also prevented tubular dysfunction after ischemia, whereas in the present study L-NAME had no protective effect. The reasons explaining those discrepancies are unknown, but they may be related to species differences in the renal response to ischemia. Our results indicate that this model of renal ischemia induces an ARF similar to that seen after total renal artery occlusion in rats, dogs, and rabbits (3, 13, 19, 25).

Postischemic ARF is characterized by acute tubular necrosis in the outer medulla mainly affecting the straight portion of the proximal tubule (the S3 segment) and less severely the medullary thick ascending limb of Henle’s loop (2, 17, 27). It appears that the hemodynamic abnormalities play an important role in the development of acute tubular necrosis by causing...
persistent regional disturbances in RBF and oxygen supply that predominantly affect the outer medulla of the kidney (11–14, 20). Impaired blood flow supply to the outer medulla after ischemic injury has been repeatedly demonstrated using different techniques, including laser-Doppler flowmetry (11–14, 30). In the present study, a partial recovery of CBF at the onset of reperfusion and a progressive decrease of CBF during the 120 min of reperfusion were observed in untreated rats, demonstrating a progressive increase in renal cortical vascular resistance. These changes are in agreement with those observed in total RBF measured with electromagnetic flowmetry. On the other hand, the alterations observed in the present study in OMBF and PBF after ischemia only partially confirmed previous reports in which a large decrease in OMBF was observed associated with a simultaneous increase in PBF, a redistribution of intrarenal blood flow that has been interpreted as the result of outer medullary congestion with the concomitant shunting of blood to vasa recta toward the inner medulla (11–14,). In our study, both OMBF and PBF signal showed a similar trend to decrease after ischemia. The reasons explaining those discrepancies are unknown, although they could be a consequence of differences in the experimental model (arterial vs. venous ischemia). Our study also shows that NAC exerts a beneficial effect by preventing the increase in total renal and outer medullary vascular resistances observed with reperfusion, thus suggesting that oxygen free radicals may play a role in the progressive renal postsischemic vasoconstriction. As indicated before, the fact that the protective effect of NAC was not observed when NO synthesis was inhibited (when L-NAME+NAC was administered) also indicates that the protection afforded by NAC seems to be related to the presence of NO, as it has been previously reported (25).

The mechanisms proposed to explain the ischemia-reperfusion renal injury include anoxia followed by release of oxygen-derived free radicals during reperfusion, leading to endothelial cell dysfunction with decreased NO release and leukocyte-endothelial adhesion and activation (2, 9, 17, 18, 24). Superoxide anion, one of these free radicals, can interact with NO to generate peroxynitrite (21), a potent and cytotoxic oxidant that could produce renal vasoconstriction and medullary ischemia, thus contributing to the persistent reduction in medullary perfusion associated with ARF. The results of the present study support this hypothesis. In our study, ischemia was followed by a significant increase in plasma levels of rhodamine 123, indicating that peroxynitrite production was significantly increased. Pretreatment with NAC prevented the increase in plasma levels of rhodamine 123 and the fall in OMBF observed in ischemic rats, thus suggesting that the beneficial effect of NAC on outer medullary circulation may be related to decreased peroxynitrite generation and/or to peroxynitrite scavenging. In addition, the fact that the beneficial effects of NAC were completely abolished in L-NAME-pretreated rats strongly suggests that these effects were dependent on the presence of NO. This interpretation is supported by the study of Caramelo et al. (3), who found that the beneficial effect of superoxide dismutase was not observed unless l-arginine was coinfused with this enzyme, leading to the hypothesis that this treatment prevented the formation of peroxynitrite by promoting NO formation and simultaneously eliminating superoxide anion (3). Therefore, the beneficial effects of NAC on the outer medullary circulation may be dependent not only on free radical scavenging but also on NO potentiation, both of them promoting vasodilatation and preventing leukocyte activation and leukocyte-endothelial adhesion and, ultimately, precluding the endothelial dysfunction associated with ischemia-reperfusion processes. Taken together, all these data indicate that NO may be beneficial during reperfusion unless enough superoxide is present to generate peroxynitrite, a very reactive free radical that seems to be responsible for at least part of the tissue damage observed after ischemia. This is consistent with the observation that both superoxide and NO must exist in equimolar concentrations to be able to generate significant amounts of peroxynitrite (21).

In the present study, plasma NO$_7$/NO$_5$ concentration has been used as index of NO generation. Our results showed that ischemia-reperfusion produced a significant increase in plasma NO$_2$/NO$_3$, confirming previous studies showing an increment in NO synthase activity during ischemia-reperfusion processes (8). On the other hand, pretreatment with L-NAME had no effect on plasma concentration of NO$_2$/NO$_3$, indicating that at the dose of L-NAME used, NO synthase activity was not inhibited significantly, perhaps due to the fact that inducible NO synthase activity increases during reperfusion and L-NAME cannot inhibit it effectively (31) However, in basal conditions, the renal function data show that this dose of L-NAME increased perfusion pressure and lowered RBF, indicating significant inhibition of NO synthase within the kidney. A similar observation has been made by Walker et al. (31), who found that at a low dose, an NO synthase inhibitor given before renal arterial ischemia was unable to reduce plasma NO$_2$/NO$_3$ concentration. Only higher doses of the inhibitor decreased plasma NO$_2$/NO$_3$ levels significantly (31). However, under the experimental conditions of our study, using high doses of L-NAME in ischemic kidneys is difficult, because the combined vasoconstriction of both maneuvers lead to complete and unrecoverable renal failure.

In summary, the results of the present study demonstrate that inferior cava vein occlusion above the renal veins is followed by an ARF associated with a progressive reduction in renal cortical, outer medullary, and papillary blood flows. Our study also shows that NAC ameliorates the deleterious effects of ICVO, a protection that appears to be related to scavenging of free radicals and its NO potentiation capabilities. This beneficial effect of NAC was more prominent on the outer medullary circulation, supporting a role for the hemodynamic disturbances in this renal circulation in the pathogenesis of postsischemic ARF.
**Perspectives**

In the past years, a number of studies have shown an association between endothelial dysfunction and the vasconstriction observed after renal ischemia. The hypothesis that the renal outer medulla plays a prominent role in the pathophysiology of those postischemic syndromes has emerged after Hellberg et al. (11) showed that the long-term outcome seems to be more dependent on the OMBF alterations than on the glomerular filtration. The outer medullary vasconstriction seems to be partially dependent on free radical generation that could determine endothelial dysfunction with a decrease and/or inactivation of NO. Further studies on the regulation of the outer medullary circulation and the mechanisms involved in the development of endothelial dysfunction in this renal circulation are needed to improve our understanding of the alterations related to the genesis of ischemic ARF.

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**REFERENCES**
