Renal ischemia induces an increase in nitric oxide levels from tissue stores

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Salom, Miguel G., Begoña Arregui, Luis F. Carbonell, Fernando Ruiz, José Luis González-Mora, and Francisco J. Fenoy. Renal ischemia induces an increase in nitric oxide levels from tissue stores. Am J Physiol Regul Integr Comp Physiol 289: R1459–R1466, 2005. First published June 9, 2005; doi:10.1152/ajpregu.00746.2004.—Tissue nitric oxide (NO) levels increase dramatically during ischemia, an effect that has been shown to be partially independent from NO synthases. Because NO is stored in tissues as S-nitrosothiols and because these compounds could release NO during ischemia, we evaluated the effects of buthionine sulfoximine (BSO; an intracellular glutathione depletor), light stimulation (which releases NO, decomposing S-nitrosothiols), and N-acetyl-L-cysteine (a sulfhydryl group donor that repletes S-nitrosothiols stores) on the changes in outer medullary NO concentration produced during 45 min of renal artery occlusion in anesthetized rats. Renal ischemia increased renal tissue NO concentration (+223%), and this effect was maintained along 45 min of renal arterial blockade. After reperfusion, NO concentration fell below preischemic values and remained stable for the remainder of the experiment. Pretreatment with 10 mg/kg nitro-L-arginine methyl ester (L-NAME) decreased significantly basal NO concentration before ischemia, but it did not modify the rise in NO levels observed during ischemia. In rats pretreated with 4 mmol/kg BSO and L-NAME, ischemia was followed by a transient increase in renal NO concentration that fell to preischemic values 20 min before reperfusion. A similar response was observed when the kidney was illuminated 40 min before the ischemia. The coadministration of 10 mg/kg iv N-acetyl-L-cysteine with BSO + L-NAME restored the increase in NO levels observed during renal ischemia and prevented the depletion of renal thiol groups. These results demonstrate that the increase in renal NO concentration observed during ischemia originates from thiol-dependent tissue stores.

voltagmetry; nitrosothiols; N-acetyl-l-cysteine; buthionine sulfoximine; photosensitive nitric oxide release

IT HAS BEEN REPORTED THAT arterial ischemia produces an abrupt and significant increase in tissue nitric oxide (NO) concentration, which can last as long as ischemia is maintained and returns to preischemic levels during reperfusion. This phenomenon has been observed in kidney (27), liver (16), heart (40), gastric tissue (15), and brain (39); although its physiological relevance is unclear, it may generate the high levels of peroxynitrite anion formed during reperfusion when a burst of superoxide anion reacts with the high levels of NO accumulated during ischemia (21), thus contributing to reperfusion damage. The mechanism responsible for these increased NO levels during renal ischemia is unknown, although it seems to be partially insensitive to NO synthesis inhibition, at least in liver and kidney (16, 27). This is not surprising because NO synthase (NOS) requires molecular oxygen. Therefore, during ischemia, NO must be released from other sources, such as tissue NO stores (22, 26, 31).

In the presence of oxygen, NO is synthesized from L-arginine through the action of NOS, and this gaseous hormone acts in the kidney by stimulating guanylyl cyclase and by inhibiting cytochrome P-450 (18). However, as soon as it is synthesized, NO avidly reacts with molecular oxygen, superoxide anion, and heme groups. The wide availability of these NO scavengers in all tissues argues against the simple diffusion-limited transport of free NO from synthase to cyclase or cytochrome. This implies that NO must be stabilized in vivo by reacting with carrier molecules that prolong its half-life and preserve its biological activity. This role may be subserved by biological molecules containing sulfhydryl groups that readily react with NO to form S-nitrosothiols (33), which are significantly more stable than NO itself and have been shown to be long-lasting and potent vasodilators. These compounds have been postulated to be biologically active intermediates in the mechanism of action of NO (32). From this point of view, it has been shown that, at physiological concentrations, NO reacts with thiol in the presence of oxygen to form S-nitrosothiol (14) and that NO circulates in mammalian plasma as nitrosothiols, mainly S-nitroso-serum albumin (32). The abundance of S-nitrosothiol in plasma compared with that of NO (3- to 4-fold) (32) suggests that plasma S-nitrosothiol may serve as a reservoir of NO, acting as an effective buffer (16). This has also been shown in vascular tissue (22), where S-nitrosothiols are known to cause a prolonged NO-dependent relaxation (31). These facts led us to hypothesize that renal ischemia induces an increase in tissue NO levels likely coming from tissue nitrosothiol stores and therefore that this phenomenon should be dependent on the presence of thiol groups in the tissue.

In the present study, it was first established that NO synthesis inhibition had no effect on the rise in tissue NO observed during renal artery occlusion. Then, because the formation of tissue S-nitrosothiols are thought to be dependent on the availability of thiol groups (3), the effect of the cellular thiol depletor buthionine sulfoximine (BSO) on the ischemia-induced increase in renal tissue NO levels was evaluated during NO synthesis inhibition. To demonstrate that the effects of BSO are specific and not due to a side effect of this compound, the actions of N-acetyl-L-cysteine (NAC), a sulfhydryl group donor, in preventing the effects of BSO were studied. Finally, because light releases NO from nitrosothiols (8, 26, 36), it was determined whether the depletion of renal

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NO stores with light stimulation before ischemia modifies the changes in NO concentration observed during ischemia.

**METHODS**

The experiments were performed on 34 anesthetized male Sprague-Dawley rats (250–300 g body wt) bred in the Animal Care Facility at the University of Murcia. 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. These experiments were approved by the University of Murcia Bioethics Committee. The rats were anesthetized with an intramuscular injection of ketamine (30 mg/kg; Rhône Merieux, Athens, GA) and an intraperitoneal injection of thiopental sodium (pentothal, 50 mg/kg; Abbott) and were then placed on a heated table to maintain body temperature at 37°C. Additional doses of thiopental sodium were given intravenously as required. A tracheotomy was performed, allowing the animals to breathe spontaneously, and cannulas were placed in the jugular vein for infusions and in the right femoral artery for measurement of arterial blood pressure. The left kidney was immobilized in a plastic holder (1, 17, 18) and maintained warm by a continuous dripping of saline at 37°C, as it is done in micropuncture experiments (6, 7). Because electrochemical NO measurements can be affected by changes in temperature, the effects of renal artery occlusion on renal temperature were evaluated in previous experiments as described (1). These experiments were performed as described above, except that a thermocouple (0.5 mm diameter) was inserted into the renal cortex, to allow for the measurement of renal temperature during renal artery occlusion. We found that a continuous dripping of warm saline maintains renal temperature during reductions of renal blood flow (1) or during renal artery occlusion.

**NO Measurements**

NO generated in the outer medulla of the left kidney was measured by differential pulsed voltammetry. We chose the renal outer medulla for NO determinations because it was previously demonstrated that the deleterious effects of renal ischemia are associated with outer medullary congestion and endothelial dysfunction observed during reperfusion (17, 28). We used a very sensitive voltammetric method (differential double normal pulse voltammetry) (10), which used a three-electrode potentiostatic system that displayed the NO sensor as the working electrode, a platinum wire as the auxiliary electrode, and an Ag/AgCl wire as the reference electrode. The following differential double normal pulse voltammetry parameters were used: potential range of 0.0–1.0 V, scan rate of 10 mV/s, prepulse 1 of 80 mV, prepulse duration of 60 ms, and pulse 2 of 80 mV × 60 ms, prepulse duration of 100 ms, and resting time of 180 ms. In these conditions, NO solutions produce an oxidation peak at 650 mV.

The NO sensor was prepared as described elsewhere (9, 25). Carbon fibers (30 μm; Textron) were glued to a copper wire with silver glue, threaded through the opening of the glass micropipettes, and sealed using epoxy resin. The electrodes were baked at 50°C for 24 h. The electrodes were then pretreated with a triangular-wave current (70 Hz, 2.5 V/30 s) followed by a constant potential (1.6 V/20 s) in PBS (pH 7.4). After that, the tips of the microelectrodes were covered with six layers of Nafion (5% in aliphatic alcohols; Sigma-Aldrich). Finally, the microelectrode tips were covered with o-phenylenediamine dihydrochloride (5 mmol/l in 0.1 M PBS), plated on the carbon surface at constant potential (+0.9 V vs. Ag/AgCl reference for 120 s). These microelectrodes are very specific for NO and very selective against other chemicals (24, 25). The specific NO oxidation current is detected at a potential of +0.65 V, and the peak height is proportional to the NO concentration in the medium. These sensors were calibrated using a NO-saturated PBS solution in anaerobic conditions at 37°C (Fig. 1). This technique has been previously validated in our laboratory to measure renal tissue NO levels (18). To measure renal NO, a small portion of the renal capsule (<1 mm²) was peeled off with microforceps, and the microelectrode was directly inserted into the renal outer medulla to a depth of 2.5 mm using a micromanipulator, with no visible bleeding (18). 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 (18). Reference and auxiliary electrodes were placed in the renal holder, in close contact with the kidney, and bathed in warm PBS (37°C) (18).

**Reduced Low-Molecular-Weight Thiol Measurements**

The reduced thiols present in the kidney were determined by the reaction with DTNB as previously described (2, 5). Briefly, frozen kidneys were homogenized in 6% TCA and centrifuged at 4,000 g for 20 min (4°C); the buffered (pH 8) supernatant was combined with DTNB (Sigma). Reduced thiol concentration was calculated on the basis of a GSH (Sigma) standard curve.

**Experimental Protocols**

The experiments were designed to study the effects of BSO, an intracellular glutathione depletor, nitro-arginine methyl ester (L-NAME), an inhibitor of NO synthesis, NAC, a sulfhydryl group donor, and light stimulation (which depletes nitrosothiols) on the changes in outer medullary NO concentration observed during a 45-min renal ischemia and a 30-min reperfusion. The rats received an intravenous infusion of saline (0.9%) at a rate of 2 ml·kg⁻¹·h⁻¹ throughout the experiment. After a 1-h equilibration period, renal outer medullary NO concentration was measured under basal conditions for 10 min. After basal NO determination, saline or L-NAME was administered intravenously, and NO was redetermined 20 min...
later for another 10-min period. After that, renal ischemia was induced by occluding the left renal pedicle with a vascular, nontraumatic clamp, to avoid the displacement of the NO electrode caused by the renal collapse, which is usually observed when only the renal artery is occluded. Forty-five minutes later, the clamp was released and the kidney was reperfused for 30 min. Renal outer medullary NO concentration was measured continuously during the ischemia and the 30-min reperfusion. Renal perfusion pressure was determined for each period. NO concentration was calculated as the means of six voltammograms (10 min) during basal and saline or L-NAME periods and as the mean of three voltammograms (5 min) thereafter. At the end of the experiment, the kidneys were removed and quickly frozen at −80°C until used to measure thiol content. Because intravascular S-nitrosothiol albumin may influence the tissue levels of S-nitrosothiols in vivo (32), the kidneys were perfused before removal with a cold PBS solution for several minutes at 120 mmHg, to eliminate all blood.

Experimental Groups

Ischemia group. These rats (n = 7) were infused with saline throughout the experiment.

Ischemia + L-NAME group. After a basal NO measurement, these animals (n = 7) received an intravenous bolus injection of L-NAME (10 mg/kg) 30 min before ischemia. This dose was chosen because it was previously shown that 1 mg/kg iv L-NAME reduces renal tissue nitrite concentration by 50% (20) and a dose of 1.8 mg/kg iv lowers renal cortical and medullary nitrite by 90 and 95%, respectively (37). In addition, we have reported that 10 mg/kg iv L-NAME decreases renal cortical NO concentration by ~80% (18).

Ischemia + BSO + L-NAME group. These rats (n = 7) were given an intraperitoneal injection of 4 mmol/kg BSO 150 min before the ischemia. It has been reported that this dose of BSO reduces by 80% the content of renal tissue sulfhydryl groups (29). One hour later, the animals were anesthetized and surgically prepared, and, after a basal determination of NO levels, an intravenous bolus injection of L-NAME (10 mg/kg) was administered 30 min before ischemia.

Ischemia + BSO + NAC + L-NAME group. These rats (n = 6) were treated with 4 mmol/kg BSO + 10 mg/kg iv NAC 150 min before ischemia. One hour later, the animals were anesthetized; after a basal determination of NO levels, an intravenous bolus injection of 10 mg/kg L-NAME was then administered 30 min before ischemia. The dose of NAC was chosen in preliminary experiments as the lower dose that restored the NO increase during ischemia in rats given BSO.

Ischemia + L-NAME + light group. To confirm that NO light releases from nitrosothiols, S-NOS-NAC was synthesized as previously reported (18) and NO was measured in vitro by introducing the NO microelectrode into a 1 mM solution of S-NOS-NAC in PBS (pH 7.4, 37°C) by normal pulse amperometry (700 mV, 100 ms, 1 Hz) before and during illumination with a cold light source (150 W). To also demonstrate that renal tissue behaves similarly during light exposure, this experiment was also performed by inserting the carbon fiber microelectrode into the kidney, illuminated with the same cold light (Fig. 5). Once it was clear that light pretreatment can release NO from renal tissue, illumination was used to deplete renal nitrosothiols. After a basal NO measurement, these animals (n = 7) received an intravenous bolus injection of 10 mg/kg L-NAME 30 min before ischemia, and the kidney was illuminated with a fiber-optic cold light source (150 W halogen lamp) to deplete nitrosothiol stores. Light was kept on for the remainder of the experiment. Again, because electrochemical NO measurements can be affected by changes in temperature, the effects of renal illumination on renal temperature were evaluated in previous experiments, as described (1). These experiments were performed as described above, except that a thermocouple (0.5 mm diameter) was inserted into the renal cortex to allow for the measurement of renal temperature during renal illumination. We found that a continuous dripping of warm saline maintains renal temperature during reductions of renal blood flow (1) or during renal illumination with a cold light source.

The reduced thiols present in the kidney were determined in the aforementioned groups. In addition, renal sulfhydryl content was also determined in sham-operated rats (n = 7) infused with saline throughout the experiment in which no ischemia was performed. Renal sulfhydryl content was also determined in the contralateral nonischemized kidneys of rats given L-NAME (Sham + L-NAME group, n = 4).

Statistical Methods

Data are presented as means ± SE. The significance of differences in the measured values was analyzed with two-way ANOVA for repeated measures followed by a Duncan’s protected t-test. The significance in the measured values of sulfhydryl groups was analyzed with a one-way ANOVA for repeated measures, followed by a Duncan’s protected t-test. Pearson’s correlation coefficient between NO concentration and renal sulfhydryl content was calculated by pooling the data from ischemia, ischemia + L-NAME, ischemia + BSO + L-NAME, ischemia + BSO + L-NAME + NAC, and ischemia + L-NAME + light groups. A value of P < 0.05 (2-tailed test) was considered statistically significant.

RESULTS

Effects of Treatments on Renal Perfusion Pressure

No differences were observed among the experimental groups in basal renal perfusion pressure values (data not shown). In the ischemia group, renal perfusion pressure remained unaltered throughout the experiment. However, renal perfusion pressure increased significantly in all groups given L-NAME (Fig. 2) and remained elevated during the experiment.

Effects of Treatments on Renal NO Levels

The changes in renal tissue NO concentrations in all groups are depicted in Figs. 3–7. No significant differences were
observed in basal NO concentrations among the experimental
groups. The administration of L-NAME was followed by a
significant decrease in NO concentration in L-NAME
(−47.2 ± 10.0%) (Fig. 4), BSO + L-NAME (−47.1 ± 5.0%),
and BSO + NAC + L-NAME groups (−38.8 ± 12.3%, Fig.
5), indicative of NO synthesis inhibition. The effect of light
on renal NO concentration can be seen in Fig. 6B, in which a
typical amperogram obtained during renal light exposure be-
fore and after NO inhibition is presented. Light stimulation
induced a striking increase in NO levels that rapidly drops
below basal levels when the light is turned off. This response
was not abolished by previous NO inhibition.

Effects of Ischemia and Reperfusion on Renal NO Levels

In Fig. 3, typical voltammograms obtained during an ische-
mia experiment in basal, ischemic, and reperfusion periods are
presented. Renal ischemia produces a striking increase in NO
levels that rapidly drops on reperfusion below basal values.

Effects of Treatments on Renal NO Concentrations

During Ischemia

These results are presented in Figs. 4, 5, and 7. In the
ischemia group, a significant and rapid increase in NO con-
centration was observed when the renal artery was clamped
(from 796.3 ± 162 nM before ischemia to 2,574.4 ± 417.9 nM
30 min after the onset of ischemia) (Fig. 4). A similar increase
in NO during renal artery occlusion was observed in L-NAME-
treated animals. However, when the animals were treated with
BSO plus L-NAME (Fig. 5), the increase in NO concentration
was blunted (1,190.5 ± 125.5 nM after 15 min of ischemia,
which then fell back to preischemic values after 25 min of
ischemia). This effect of renal glutathione depletion was not
observed when the thiol group donor NAC was coadministered
with BSO (NO rose to a maximum of 2,882.7 ± 385.0 nM at
25 min of ischemia and only fell during reperfusion). In the
L-NAME + light group (Fig. 7), a rapid and brief increase in
NO concentration was observed after ischemia (3,246.7 ±
1,005.1 and 990.4 ± 327.5 nM, 15 and 30 min after ischemia,
respectively). Subsequently, NO concentration fell in light-
treated rats below the values observed in control rats during the
late ischemia periods.

Effects of Treatments on the Renal NO Concentration

During Reperfusion

When kidneys were reperfused, NO concentration fell
quickly below preischemic values in the ischemia group until
the end of the experiment (Figs. 4, 5, and 7). No significant
differences were observed among groups along reperfusion.

Effects of BSO and NAC on Renal Sulfhydryl Group Content

The renal sulfhydryl content in sham-operated and sham-
operated + L-NAME rats and also in ischemic kidneys of rats
given saline, L-NAME, BSO + L-NAME, BSO + NAC +
L-NAME, and L-NAME + light are presented in Fig. 8.
ischemia decreased renal sulfhydryl content (from 0.840 ± 0.201 in sham-operated to 0.420 ± 0.073 μmol/g wet weight in ischemic rats). In ischemic rats given BSO + l-NAME or l-NAME + light, the observed fall in renal thiol content was greater than that after ischemia alone (0.107 ± 0.021 and 0.083 ± 0.015 μmol/g wet wt, respectively). The renal depletion of sulfhydryl groups produced by BSO + l-NAME was partially prevented (0.226 ± 0.034 μmol/g wet wt) when the animals were pretreated with the thiol group donor NAC. A significant correlation (r = 0.731, P < 0.001) was observed between renal sulfhydryl content at the end of the experiment and outer medullary NO concentration after 45 min of ischemia (Fig. 9). L-NAME alone had no effect on renal sulfhydryl content in sham-operated or in ischemic kidneys.

**DISCUSSION**

In the present study, renal artery occlusion caused an important increase in renal tissue NO concentration, reaching a plateau after 20–25 min of ischemia that was maintained until reperfusion. Our results also show that NO synthesis inhibition did not prevent this increase in NO concentration. However, this rise in NO during ischemia was blunted by sulfhydryl group depletion with BSO or S-nitrosothiol depletion with light stimulation. This effect of BSO appears to be specific because it was prevented by replenishing renal tissue thiol group levels with NAC, a sulfhydryl group donor.

The renal tissue basal NO concentrations observed in the present study were in the nanomolar range, similar to those previously reported in renal cortex (4, 18). However, the inhibition of NO synthesis only produced a ~50% decrease in basal outer medullary NO concentration, an effect that is smaller than the 80% drop previously reported in renal cortex (18). The smaller drop of outer medullary NO concentration...
could indicate a partial NO synthesis inhibition. However, this apparent lack of effect of $\text{l}$-NAME in the renal medulla has been previously reported, and it has been attributed to the fact that this region of the kidney is physiologically hypoxic, and NO synthesis inhibition lowers medullary blood flow and PO$_2$ even more. This has been postulated to account for the maintenance of NO levels despite the NO synthesis blockade because NO degradation should be reduced in the near anaerobic conditions originated (12). There are reports indicating that $\text{l}$-NAME, in the dosage range used in the present study, is effective in inhibiting renal NO generation. Matsuda et al. (20) showed that a 1 mg/kg iv dose of $\text{l}$-NAME induced a sustained reduction of >50% in cortical and medullary nitrite concentrations. Also, Walkowska et al. (37) found that a dose of 1.8 mg/kg iv inhibited NO synthesis by 90 and 95%, respectively, in renal cortex and medulla. In addition, we believe that NO synthesis inhibition lasts as long as the experiment because the increase in renal perfusion pressure seen after $\text{l}$-NAME was maintained (see Fig. 2).

The effect of ischemia increasing NO levels has already been observed in previous studies performed in a variety of organs. Zweier et al. (40) observed a 10-fold increase in NO in the ischemic heart. More recently, Lhuillier et al. (16) described a twofold increase in NO in the ischemic liver that was insensitive to NOS inhibition. In the present study, renal ischemia was followed by an immediate threefold increase in NO concentration, reaching a maximum 25 min after the onset of the ischemia. The short delay between the renal artery occlusion and the increase of the NO signal may be consistent with generation from NOS. However, although NOS inhibition with $\text{l}$-NAME induced a significant decrease in basal NO concentration in preischemic kidneys and a long-lasting increase in arterial pressure, $\text{l}$-NAME alone failed to prevent the increase in NO observed during ischemia. This is not surprising because the reaction catalyzed by NOS requires molecular O$_2$ (19) and during ischemia, the oxygen supply to the kidney is interrupted. Thus the persistence of this ischemia-induced NO rise in renal tissue despite NOS inhibition suggests the presence of enzyme-independent sources of NO, such as NO stores, that are known to be available in tissues (22, 26, 31) and could release NO and maintain NO levels in anaerobic conditions. In this regard, because of the very short half-life of NO, it has been hypothesized that the pathways from NO synthesis to the target enzymes (guanylyl cyclase, cytochrome P-450) must include the formation of $S$-nitrosothiols and/or $S$-nitroso-proteins, which are potential enzyme-independent sources of NO that store and release the gas in tissues. It has been reported that NO stores are available in tissues as $S$-nitrosothiols, which are stored in cells as $S$-nitrosoglutathione (38). We therefore hypothesized that renal tissue nitrosothiols release NO when renal blood flow is blocked, oxygen tension falls to zero, and NO synthesis is abrogated. In this situation, as $S$-nitrosothiols decompose slowly to release NO (31), and if this NO released is not inactivated by oxygen or oxyhemoglobin, NO concentration should increase progressively to reach a maximum. The fact that $\text{l}$-NAME did not modify renal thiol content may also explain why the ischemia-induced increase in NO levels is not influenced by previous NO synthesis inhibition alone because this rise in NO should last until NO stores are depleted. In the ischemic kidneys of rats given saline, the increase of NO concentration was maintained throughout the duration of ischemia, and renal sulfhydryl groups were reduced by only 48.1%, indicating that cellular NO stores were not depleted during 45 min of renal pedicle occlusion. This is compatible with previous reports indicating half lives of cellular $S$-nitrosothiol stores ranging from 40 to 180 min (31, 33).

If intracellular $S$-nitrosothiol stores are the enzyme-independent source increasing NO during ischemia, this rise in NO should be blunted if $S$-nitrosothiol stores are reduced by previously depleting the tissue thiol group content. In the present study, we determined the effect of renal tissue thiol depletion caused by BSO, a glutathione depletor that induces a rapid decrease in tissue GSH due to specific inhibition of $\gamma$-glutamylcysteine synthetase (11), and renal exposure to a cold light source, known to release NO by decomposing nitrosothiols (8, 26, 36). We observed a 74.5% decrease in the renal concentration of sulfhydryl groups 150 min after BSO injection, which is similar to values previously reported (29), and an 80.4% decrease after 90 min of cold light exposure. As expected, this thiol group depletion caused a transient increase in NO during ischemia because NO levels fell to near basal values after 25 min of ischemia, well before reperfusion, which is compatible with the hypothesis that the NO stores were low and were totally depleted after 25 min of ischemia. This point of view is also compatible with the fact that NAC, a sulfhydryl group donor, prevented the effects of BSO on renal thiol group levels and also on the changes in NO levels observed during ischemia. The fact that a positive and significant correlation between renal sulfhydryl content and outer medullary NO concentration after 45 min of ischemia was observed also supports this interpretation. Although the dose of NAC used does not normalize renal thiol group levels (Fig. 8), it seems that it increases those levels above certain threshold necessary to maintain enough nitrosothiol stores to release NO during ischemia; in the groups where thiols fall below that threshold.
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(treated with BSO or light), NO concentrations cannot be maintained during the clamping of the renal pedicle.

It has been shown that S-nitroso-serum albumin accounts for most of all RSNO in vivo (32). Thus plasma proteins can be a source of sulfhydryl groups. To minimize the impact of this potential source of sulfhydryl groups in our measurements, kidneys were removed after they were perfused with a cold PBS solution for several minutes under 120 mmHg perfusion pressure. In addition, renal homogenates were deproteinized with TCA, thus ensuring that only low-molecular-weight thiols were measured. However, the possible release of NO from intravascular nitrosothiols during renal ischemia cannot be excluded.

Cellular NO stores have been previously shown to be photosensitive S-nitrosothiols, compounds that release NO under light stimulation and become depleted after a few minutes (8, 26, 36). This effect is depicted in Fig. 6A, in which it is shown that light stimulation releases NO from a S-NO-NAC solution. In addition, the fact that renal tissue also releases NO during light exposure is compatible with the idea that NO is stored as light-sensitive nitrosothiols in the kidney. Therefore, a prolonged light stimulation before ischemia, combined with NOS inhibition, should deplete these renal NO stores and blunt the increase in NO levels during ischemia. The results of the present experiments show that a 40-min light-induced nitrosoothiol depletion before ischemia caused NO to increase only transiently during ischemia, falling to presischemic levels after only 30 min of ischemia, well before reperfusion. This effect was very similar to that observed in rats given BSO, thus suggesting that light stimulation produced depletion of renal NO stores and further supporting the idea that these thiol-related stores are the source of the originating rise in NO during renal ischemia. However, in contrast with BSO, light stimulation did not blunt the initial increase in NO concentration observed after renal occlusion. This different NO response might reflect the existence of a photosensitive NO store that releases NO on renal occlusion. In this regard, it has been previously reported that there is a nonenzymatic NO source that releases NO on renal occlusion. This different NO response might reflect the existence of a photosensitive NO store that releases NO on renal occlusion. This nonenzymatic NO source is thought to occur on reperfusion, when superoxide is generated by the restoration of renal blood flow with the reperfusion of the renal pedicle.

When the kidneys were reperfused, NO concentration fell transiently during ischemia, falling to preischemic levels after ischemia (21). Peroxynitrite is a potent and cytotoxic oxidant that may contribute to the renal vasoconstriction and medullary damage produced during reperfusion. In addition, it has been shown that the early response to ischemic preconditioning, an adaptive pathophysiological phenomenon that increases the tolerance to ischemic-reperfusion injury, is abolished by NOS blockade and can be reproduced by the administration of a NO donor (35), suggesting that the induced tolerance to ischemia is related to NO production. Additional studies are needed to gain a better understanding on the pathophysiological role of NO in the complex relationships of phenomena taking place during ischemia and reperfusion.

In summary, the results of the present study show that renal ischemia is followed by a rapid increase in intrarenal NO concentration that is maintained until reperfusion, when a fast drop in NO levels near preischemic values is observed. The increased NO concentration observed seems to be independent of NOS and appears to originate in tissue NO stores that release NO during ischemia.

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