Renal Responses to Intra-arterial Administration of Nitric Oxide Donor in Dogs

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Inhibition of nitric oxide synthesis by intra-arterial administration of nitro-L-arginine (NLA) leads to attenuation of the slope of the relation between renal arterial pressure (RAP) and sodium excretion without an alteration in renal autoregulatory efficiency. In the present study, we examined whether only the presence of nitric oxide or, alternatively, changes in nitric oxide production during changes in RAP are required for pressure natriuresis to occur. Anesthetized sodium-replete dogs (n=8) were treated with NLA (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$) to inhibit endogenous nitric oxide formation, and *S*-nitroso-*n*-acetylpenicillamine (SNAP) was infused intra-arterially at a constant rate (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) to replenish intrarenal nitric oxide levels. Renal responses to reductions in RAP within the autoregulatory range were assessed before and during NLA infusion followed by SNAP+NLA infusion. As reported previously, NLA infusion alone increased renal vascular resistance and decreased renal blood flow, urine flow, sodium excretion, and fractional excretion of sodium, with no change in glomerular filtration rate. Autoregulatory efficiency remained intact, whereas the pressure-induced natriuretic responses were attenuated. During SNAP+NLA infusion, renal blood flow increased from 2.8±0.3 to 3.5±0.3 $mL \cdot min^{-1} \cdot g^{-1}$ ($P<.001$), without significant changes in glomerular filtration rate (0.75±0.07 to 0.81±0.05 $mL \cdot min^{-1} \cdot g^{-1}$); the autoregulatory efficiency of renal blood flow and glomerular filtration rate remained intact. SNAP increased urine flow (4.8±1.8 to 10.0±2.5 $\mu L \cdot min^{-1} \cdot g^{-1}$), sodium excretion (0.63±0.26 to 1.70±0.37 $\mu mol \cdot min^{-1} \cdot g^{-1}$), and fractional excretion of sodium (0.55±0.20% to 1.38±0.27%). Despite the natriuresis induced by SNAP, the slope of the relation between sodium excretion and RAP remained attenuated. These data support the concept that alterations in intrarenal nitric oxide production during changes in RAP participate in the mediation of pressure natriuresis. (*Hypertension*. 1993;22:535-541.)

KEY WORDS • hemodynamics • autoregulation • arginine • nitric oxide • natriuresis

Recent studies have implicated a role of nitric oxide (NO) as an important regulator of renal hemodynamics and excretory function.[1-12] NO is synthesized from the amino acid L-arginine by mammalian tissues including vascular endothelium. The enzymatic synthesis of NO can be competitively inhibited by structural analogues of L-arginine such as N^G-monomethyl L-arginine and nitro-L-arginine (NLA).[13] The formation and release of NO can also be enhanced by agents such as acetylcholine, bradykinin, and other agonists.[14] The role of NO in the control of renal function has been assessed primarily with the use of pharmacologic interventions to stimulate NO formation and release or to block the NO synthesis. Whereas acetylcholine and bradykinin exert part of their renal effects by enhancing NO activity in the kidney,[5,6,8] other mechanisms may also contribute to the effects of these agents.[5,6,8,14,15] For the evaluation of the unique and specific actions of NO on renal hemodynamics and function, agents commonly termed nitrovasodilators, known to release NO in biologic fluids,[16,17] can be used.

Nitrovasodilators have been used clinically for about 100 years and are still widely used in conditions such as angina pectoris, hypertensive emergencies, and pulmonary hypertension.[18] Among such nitrovasodilators, the commonly known agents are sodium nitroprusside, glyceryl trinitrate, and *S*-nitrosothiol compounds. The available evidence indicates that the final common effector molecule of all nitrovasodilators is NO, which then activates soluble guanylate cyclase.[17] *S*-Nitrosothiols are potent vasodilators and have been used to mimic the actions of endogenously formed endothelium-derived NO.[16,17,19-21]

There is growing evidence that NO may also exert direct effects on urinary sodium excretion ($U_{Na}V$).[2,7-9,11] It has been demonstrated that inhibitors of NO synthesis, when administered intrarenally, elicit a diminution of $U_{Na}V$.[8,9,11] On the other hand, stimulators of NO formation such as acetylcholine and bradykinin cause diuresis and natriuresis.[5,6,22] These effects are not consistently associated with appreciable changes in glomerular filtration rate (GFR), indicating that NO exerts a tubular effect, either directly or indirectly, to regulate sodium transport. In contrast, it has also been reported that systemic bolus administration of NO synthesis inhibitors in rats[2] results in diuresis and natriuresis. The reason for such different responses is not yet clearly

Received January 21, 1993; accepted in revised form June 11, 1993.

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Presented in part at the Experimental Biology Meeting, New Orleans, La, March 28-April 1, 1993, and at the Inter-American Society of Hypertension Meeting, La Jolla, Calif, April 25-29, 1993.

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understood but could be due to the influence of the associated increase in arterial pressure[4] or other mechanisms that are activated by systemic administration of L-arginine analogues. Nevertheless, inhibition of NO synthesis by intrarenal administration of NLA leads to a marked attenuation of the urine flow and sodium excretory responses to changes in renal arterial pressure (RAP) without any effect on the autoregulatory capability of renal blood flow (RBF) and GFR, suggesting that NO exerts either a permissive or a mediatory role in pressure natriuresis.[9,11]

The present investigation was carried out to determine whether simply the presence of NO in contrast to a change in NO production during changes in RAP is required for pressure natriuresis to occur. For this study, we used *S*-nitroso-*n*-acetylpenicillamine (SNAP), an *S*-nitrosothiol known to be a potent in vivo vasodilator.[16] SNAP was infused intrarenally at a constant rate in anesthetized dogs in which endogenous NO synthesis was inhibited by the administration of NLA. The objective was to achieve a relatively constant level of intrarenal NO activity during changes in RAP under conditions in which the renal capability to form endogenous NO was blocked.

Methods

Experiments were carried out in eight mongrel dogs (20.8±0.6 kg body wt) of either sex. The preparation of the animals and basic experimental techniques are similar to those previously described.[9] To achieve a positive sodium balance and stimulate a physiological natriuresis, we added supplemental amounts of sodium chloride (1.5 g/kg body wt per day for 3 days) to the normal laboratory diet. On the morning of the experimental day, the dogs were anesthetized with pentobarbital sodium (30 mg/kg body wt IV); surgical anesthesia was maintained throughout the experiment by additional doses of pentobarbital sodium as required (approximately 4 $mg \cdot kg^{-1} \cdot h^{-1}$). A cuffed endotracheal tube was inserted into the trachea to allow positive pressure ventilation with an artificial respirator at a stroke rate of 18/min and stroke volume of approximately 15 mL/kg body wt. Body temperature of the animal was measured continuously by a telethermometer placed in the rectum and was maintained within the normal range with an electric heating pad placed under the dog.

Systemic arterial pressure of these dogs was measured from a catheter placed in the abdominal aorta inserted via the right femoral artery and connected to a pressure transducer and was recorded on a polygraph (model 7D, Grass Instrument Co, Quincy, Mass). At least 30 minutes before the experimental protocol was started, dogs were subjected to occlusion of the right common carotid artery with partial constriction of the left common carotid artery in order to elevate the basal level of arterial pressure to approximately 150 mm Hg. This procedure allowed evaluation of the relation between renal perfusion pressure and $U_{Na}V$ over a wider range of arterial pressure. The left femoral artery was cannulated for collection of blood samples. The femoral and jugular veins were cannulated for administration of an inulin solution and additional doses of pentobarbital sodium as necessary. During the entire experimental period, dogs were given a continuous infusion of isotonic sodium chloride solution (0.9%) at a rate of 0.025 $mL \cdot min^{-1} \cdot kg^{-1}$ via a catheter placed in the right femoral vein.

The left kidney was exposed through a flank incision, and the renal artery was isolated from surrounding tissue. The kidney was denervated by cutting the renal nerves. RBF was measured with an electromagnetic flow probe placed on the renal artery near its origin from the aorta and connected to a square wave flowmeter (Carolina Medical Electronics, King, NC). The flow traces were recorded on the polygraph, and zero-flow baseline was determined at the beginning and end of each experiment by momentarily occluding the artery. An adjustable plastic clamp was placed around the renal artery distal to the flow probe to achieve reductions in RAP. A curved 23-gauge needle cannula was inserted into the renal artery distal to the plastic clamp and was connected to another pressure transducer with a polyethylene catheter to measure RAP. Another catheter was also connected to this needle cannula for continuous infusion of heparinized saline at a rate of 0.4 mL/min to prevent any clot formation and to allow intrarenal administration of drugs (NLA and SNAP). Drugs were dissolved in the heparinized saline, and the concentrations were adjusted to maintain the same volume infusion rate (0.4 mL/min). Urine was collected into a graduated test tube from a catheter placed in the ureter.

After completion of all surgical procedures, a 2.5% solution of inulin in normal saline was administered via a catheter placed in the left jugular vein. A priming dose of 1.6 mL/kg body wt of inulin solution was followed by a sustaining infusion of 0.03 $mL \cdot min^{-1} \cdot kg^{-1}$ body wt. At least 45 minutes was allowed between the initiation of the inulin infusion and the start of control hemodynamic measurements and urine collections. The experimental protocol started with urine collections for two consecutive 10-minute periods at a spontaneous RAP of approximately 150 mm Hg. At the midpoint of each urine collection period, an arterial blood sample (2 mL) was taken to measure plasma inulin, sodium, and potassium concentrations. After control measurements at spontaneous arterial pressure, step reductions in RAP (approximately 125, 100, and 75 mm Hg) were produced by adjusting the clamp. At each level of RAP, at least 5 minutes was allowed for stabilization before a 10-minute urine collection was made. Below 75 mm Hg of arterial pressure, RAP was further reduced in steps of 15 to 20 mm Hg for 2 to 3 minutes in each step until RBF was reduced to near zero. After the last reduction in RAP, the clamp was released completely to reestablish control RAP and RBF. After control measurements, a continuous infusion of NLA (Aldrich Chemical Co Inc, Milwaukee, Wis; dissolved in heparinized normal saline, wt/vol; pH 6.8±0.3) was initiated at a rate of 50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ intrarenally. This dose of NLA was previously reported to yield an effective blockade of endogenous NO activity in the kidney.[8] Thirty minutes after the initiation of the NLA infusion, the same protocol was repeated to examine pressure-related responses during NO synthesis inhibition.

After reestablishment of the control RAP and RBF at the end of the experimental protocol with NLA infusion, a continuous intrarenal infusion of SNAP (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$; provided by Dr Louis J. Ignarro, De-

Renal Responses to Intra-arterial Infusion of *S*-Nitroso-*n*-Acetylpenicillamine in Dogs (n=8) Treated With Nitro-L-Arginine

Parameter	Control	NLA	SNAP+NLA
Arterial pressure, mm Hg	152±4.6	159±4.8	149±5.4*
RVR, mm Hg · mL^{-1} · min^{-1} · g^{-1}	36.7±2.4	57.6±3.8†	43.5±3.3*
RBF, mL · min^{-1} · g^{-1}	4.14±0.38	2.76±0.26†	3.50±0.32*
GFR, mL · min^{-1} · g^{-1}	0.86±0.06	0.75±0.07	0.81±0.05
Urine flow, μL · min^{-1} · g^{-1}	16.1±5.3	4.8±1.8†	10.0±2.5*
$U_{Na}V$, μmol · min^{-1} · g^{-1}	3.00±0.72	0.63±0.26†	1.70±0.37*
FE_{Na}, %	2.24±0.43	0.55±0.20†	1.38±0.27*
U_KV, μmol · min^{-1} · g^{-1}	0.57±0.14	0.38±0.08†	0.73±0.12*

NLA indicates nitro-L-arginine; SNAP, *S*-nitroso-*n*-acetylpenicillamine; RVR, renal vascular resistance; RBF, renal blood flow; GFR, glomerular filtration rate; $U_{Na}V$, urinary sodium excretion; FE_{Na}, fractional excretion of sodium; and U_KV, urinary potassium excretion. Data are expressed as mean±SEM.

*$P<.05$ vs NLA.

†$P<.05$ vs control.

partment of Pharmacology, University of California, Los Angeles) was added to the NLA infusion. This SNAP dose was used for this study because it was observed that a higher dose usually caused substantive decreases in systemic arterial pressure that prevented evaluation of renal responses to the higher levels of RAP. Both solutions (SNAP and NLA) were mixed and infused in a single syringe, and the drug concentrations were adjusted to maintain the same rate of volume infusion (0.4 mL/min). SNAP was dissolved in 0.9% sodium chloride solution (pH 6.86±0.04). Maximal stability of the SNAP solution was maintained by placing an ice jacket around the syringe during continuous intrarenal infusion.[16] Fifteen minutes after the initiation of combined SNAP and NLA infusion, the protocol was repeated to examine the renal responses to reductions in RAP in the presence of exogenous NO administration.

The effects of intra-arterial infusion of the cold solution of saline vehicle on renal hemodynamics and renal excretory values were assessed in three NLA-treated dogs. After two consecutive 10-minute urine-collection periods with intrarenal infusion of vehicle at room temperature, a cold solution of the saline vehicle (syringe jacketed with ice as in SNAP infusion) was initiated. Fifteen minutes after the initiation of the cold solution, another set of 10-minute urine samples was collected. It was observed that infusion of cold saline intrarenally at the low infusion rate used to deliver the drugs did not perceptibly change the basal level of renal parameters measured in this study.

At the end of each experiment, the flow probe was calibrated in situ by collection of timed blood samples at different flow rates into a graduated cylinder from a catheter placed in the renal artery. The kidney was then removed, stripped of all surrounding tissue, blotted dry, and weighed so that the calculated values could be expressed per gram of net kidney weight. Flame photometry (Instrumentation Laboratories, Lexington, Mass) was used to determine the sodium and potassium concentrations in plasma and urine. Inulin concentrations in plasma and urine samples were determined by the anthrone colorimetric technique. GFR was calculated with standard inulin clearance techniques. Microhematocrit measurements were performed on all arterial blood samples. Values are reported as mean±SEM. Statistical comparisons of differences in the responses were conducted with analysis of variance for repeated measures followed by the Newman-Keuls test. Differences in the mean values were deemed significant at a value of $P \leq .05$. The RBF autoregulation curve was generated by extrapolating the values of RBF at different levels of RAP (at 25 mm Hg intervals ranging from 150 to 25 mm Hg). Two separate linear regression analyses of the pressure-flow relations were carried out in each dog to obtain the extrapolated values: at the pressure levels at which RBF was autoregulated and at lower pressures at which a linear relation between RAP and RBF was obtained. RBF was considered autoregulated when the RBF values remained within 5% of the control RBF. The RAP versus RVR relation curve was also generated by extrapolating the values of RVR at different levels of RAP as in autoregulation curves.

Results

After stabilization of the preparation, the mean values of plasma sodium, potassium, and hematocrit during the control periods were 147±1.4 mmol/L, 3.5±0.1 mmol/L, and 43.7±2.7%, respectively. These values did not change significantly during infusion of NLA (147±1.3 mmol/L, 3.6±0.2 mmol/L, and 42.3±3.4%) or SNAP+NLA (148±1.4 mmol/L, 3.7±0.1 mmol/L, and 42.1±3.5%).

Effect of SNAP Infusion on Basal Level of Renal Hemodynamics and Function in NLA-Treated Dogs

The Table summarizes the results obtained in the eight study dogs. Control arterial pressure was elevated to 152±5 mm Hg because of the partial constriction of the carotid arteries. This effect waned slightly during the course of the experiment but returned to this range during NLA infusion (159±5 mm Hg). NLA infusion alone resulted in an increase of 58±7% in RVR and decreases of 33±3% in RBF, 68±5% in urine flow, 84±4% in $U_{Na}V$, 80±5% in fractional excretion of sodium (FE_{Na}), and 31±8% in urinary potassium excretion (U_KV). There was a slight but statistically insignificant decrease in GFR (−12±7%) during NLA administration. These findings

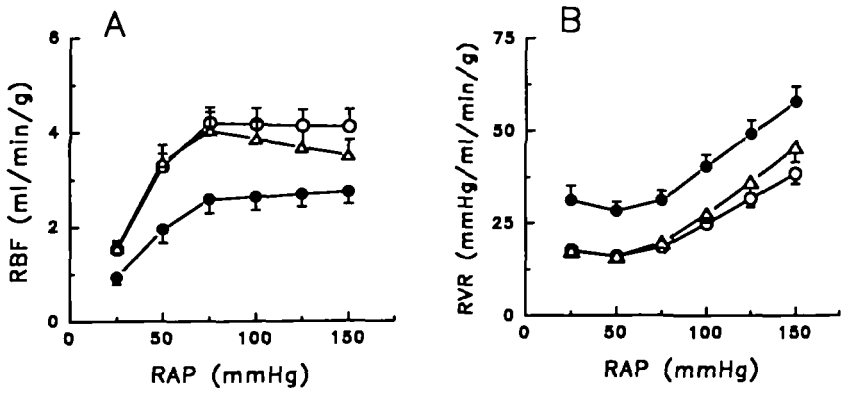

FIG 1. *Line graphs showing renal blood flow (RBF) (A) and renal vascular resistance (RVR) (B) responses to acute reductions in renal arterial pressure (RAP) before* (○) *and during* (●) *intrarenal infusion of nitro-L-arginine (NLA) and during* (△) *infusion of S-nitroso-n-acetylpenicillamine (SNAP)+NLA (n=8). Mean curves for blood flow autoregulation and RAP vs RVR relation were generated by extrapolating values of RBF and RVR at different levels of RAP from each individual curve obtained in each dog (detailed in "Methods"). Error bars indicate SEM.*

are similar to our earlier reported observations in salt-replete anesthetized dogs.[9]

Addition of SNAP (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) to the intra-arterial infusion line for more than 15 minutes partially reversed the effects caused by infusion of NLA alone. Systemic arterial pressure and RAP decreased gradually starting 3 minutes after the onset of the SNAP infusion. Administration of SNAP for more than 15 minutes led to decreases in systemic arterial pressure from 159±5 to 149±5 mm Hg ($P<.05$). RBF increased consistently and started to increase within 2 minutes of infusion and reached its near maximum peak within 5 minutes of SNAP infusion. The Table summarizes the results obtained at steady state after combined SNAP and NLA infusion for 15 minutes. As noted in the Table, during combined infusion there were significant decreases in RVR and increases in RBF, $U_{Na}V$, FE_{Na}, and U_KV from the levels obtained during NLA infusion alone. The slight increase in the mean value of GFR during addition of SNAP from the mean value during infusion of NLA alone was not statistically significant. Comparatively larger relative increases in U_KV than $U_{Na}V$ during SNAP infusion were also noted in these dogs.

Effect of SNAP Infusion on Renal Autoregulation

Figs 1 and 2 illustrate the effect of SNAP infusion on autoregulatory efficiency of RBF and GFR in NLA-treated dogs. As reported earlier,[8,9] autoregulatory efficiency of RBF and GFR remained intact during NLA infusion alone; however, there was a leftward shift of the RAP versus RVR relation curve during NLA infusion (Fig 1B) due to increases in RVR to NLA treatment, as reported previously. The autoregulation plateau of RBF was lowered, without any significant change in the slope of the autoregulatory portion of the curve during infusion of NLA alone (Fig 1A).[8,9] Also, the slope of the linear portion of the RBF autoregulation curve below 75 mm Hg was slightly decreased during NLA treatment (0.07±0.01 to 0.04±0.01 $mL \cdot min^{-1} \cdot mm\ Hg^{-1}$; $P<.01$). GFR remained well autoregulated at a mean RAP above 75 mm Hg in NLA-treated dogs (Fig 2A), without any significant change in the slope of the relation between RAP and GFR.[8,9]

After administration of SNAP to NLA-treated dogs, the basic pattern of autoregulatory efficiency of RBF and GFR remained intact (Figs 1 and 2). The RBF autoregulation plateau was partially restored toward control level during SNAP treatment. Interestingly, RBF autoregulatory efficiency was slightly augmented, with slight increases in RBF at the lower arterial pressures during SNAP infusion. As a result, there was a slight but significant change in the mean slope of the autoregulatory portion of the curves from the NLA infusion period to the SNAP+NLA infusion period (0.002±0.01 to −0.007±0.001 $mL \cdot min^{-1} \cdot g^{-1} \cdot mm\ Hg^{-1}$; $P<.01$). The slopes of the linear portion of the curves at RAP below 75 mm Hg increased during SNAP treatment compared with the NLA infusion period (0.08±0.01 $mL \cdot min^{-1} \cdot g^{-1} \cdot mm\ Hg^{-1}$; $P<.01$). However, the autoregulatory efficiency of GFR at a mean RAP above 75 mm Hg remained intact during SNAP treatment, as in both control and NLA treatment periods (Fig 2A). As SNAP administration caused reductions in RVR, the RAP versus RVR relation curve during the NLA treat-

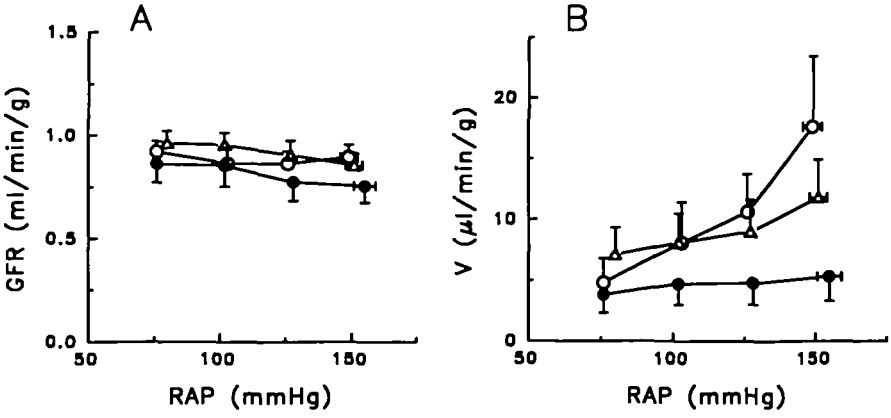

FIG 2. *Line graphs showing glomerular filtration rate (GFR) (A) and urine flow (V) (B) responses to acute reductions in renal arterial pressure (RAP) above 75 mm Hg before* (○) *and during* (●) *nitro-L-arginine (NLA) infusion and during* (△) *infusion of S-nitroso-n-acetylpenicillamine (SNAP)+NLA (n=8). Error bars indicate SEM.*

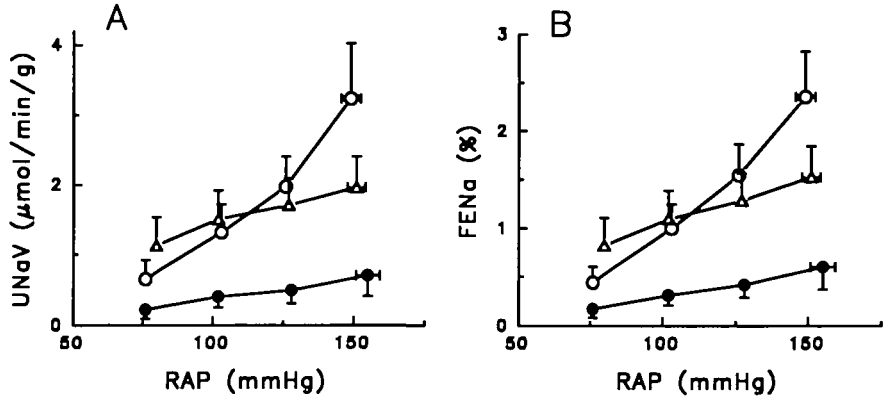

FIG 3. *Line graphs showing sodium excretion (UNaV) (A) and fractional excretion of sodium (FENa) (B) responses to acute changes in renal arterial pressure (RAP) above 75 mm Hg before* (○) *and during* (●) *nitro-L-arginine (NLA) infusion and during* (△) *infusion of S-nitroso-n-acetylpenicillamine (SNAP)+NLA (n=8). Responses to reductions in RAP remained attenuated during SNAP infusion as during NLA infusion. Error bars indicate SEM.*

ment period shifted to the right toward the control curve during the SNAP period (Fig 1B).

Effect of SNAP on Renal Excretory Responses to Reductions in Renal Arterial Pressure

Fig 2 illustrates the effects of SNAP infusion on pressure-induced changes in excretory function of the kidney. As reported earlier,[8,9] there was a marked attenuation of urine flow (Fig 2B), $U_{Na}V$ (Fig 3A), and FE_{Na} (Fig 3B) responses to changes in RAP during NLA treatment. The slope of the RAP versus urine flow relation was significantly reduced, from 0.16±0.05 to 0.014±0.01 $\mu L \cdot min^{-1} \cdot g^{-1} \cdot mm\ Hg^{-1}$ ($P<.01$), during NLA infusion alone. The slopes of the RAP versus $U_{Na}V$ and RAP versus FE_{Na} relations were also reduced, from 0.03±0.01 to 0.01±0.002 $\mu mol \cdot min^{-1} \cdot g^{-1} \cdot mm\ Hg^{-1}$ ($P<.01$) and from 0.03±0.005 to 0.004±0.002 $\% \cdot mm\ Hg^{-1}$ ($P<.01$), respectively, during NLA infusion. These responses are consistent with our earlier reported observations in sodium-replete dogs.[9]

After addition of SNAP, the values of urine flow, $U_{Na}V$, FE_{Na}, and U_KV at a spontaneous level of RAP increased from the values during NLA infusion alone (Table). Nevertheless, the excretory responses to reductions in RAP remained attenuated, as was observed during NLA treatment alone (Figs 2 and 3). There were no significant changes in the slopes of RAP versus urine flow (0.014±0.010 to 0.04±0.013 $\mu L \cdot min^{-1} \cdot g^{-1} \cdot mm\ Hg^{-1}$) and RAP versus $U_{Na}V$ (0.01±0.002 to 0.01±0.003 $\mu mol \cdot min^{-1} \cdot g^{-1} \cdot mm\ Hg^{-1}$) during addition of SNAP. The slope of RAP versus FE_{Na} also remained the same as during NLA infusion alone (0.004±0.002 to 0.008±0.002 $\% \cdot mm\ Hg^{-1}$). Thus, the slope of the pressure-natriuresis relation remained markedly attenuated even though the absolute excretion rates were elevated by the SNAP.

Discussion

The present investigation demonstrates that direct infusion of the NO donor SNAP into the renal artery in anesthetized dogs in which endogenous NO synthesis was inhibited with NLA elicited vasodilator and natriuretic responses in the kidney. There were decreases in RVR and increases in RBF, urine flow, and $U_{Na}V$, without significant changes in GFR. We have also observed that these responses to SNAP administration were dose dependent.[23] As NO generation is the essential effector of the mechanism of action of nitrovasodilators,[24] and the biologic actions of *S*-nitrosothiols such as SNAP are attributed to NO,[16] the renal responses to SNAP infusion observed in these experiments are most likely due to the intrarenal actions of NO. *S*-Nitrosothiols were previously proposed to be active intermediates in mediating the vascular smooth muscle relaxant actions of nitrovasodilators.[16] However, it has been shown that nitrovasodilators generate NO,[24] and such generation is responsible for the activation of soluble guanylate cyclase.[25] As *S*-nitrosothiols are very unstable, especially at physiological pH and temperature, and spontaneously release NO,[16] and activation of guanylate cyclase by NO can occur without the presence of thiols,[17] it is now believed that NO is the final active principle in the mechanism of actions of *S*-nitrosothiols in guanylate cyclase activation.[17,19-21] It has been shown recently that vasodilator responses to SNAP and NO are inhibited in a similar manner in vivo by methylene blue, an inhibitor of soluble guanylate cyclase, indicating that SNAP releases NO and activates soluble guanylate cyclase.[26]

The basal level of arterial pressure was elevated to approximately 150 mm Hg by partial occlusion of the carotid arteries in these experiments. This procedure might influence the subsequent responses to NLA and SNAP. However, because the kidney was denervated, it is unlikely that the observed renal responses to NLA and SNAP infusion were influenced by neurohumoral factors that could appear as a consequence of carotid occlusion. Previous studies have indicated that the secretion of catecholamines (epinephrine and norepinephrine) from the adrenal medulla was relatively unaffected by bilateral carotid occlusion in conscious dogs.[27]. It also seems unlikely that there were direct interactions between catecholamine release and NO in modulating renal responses to NO donor infusion. Hypotension produced by either hemorrhage or infusion of nitroglycerin (a nitrovasodilator) induced similar effects on heart rate and arterial pressure in conscious dogs.[27] Although the neural modulation of NO activity is a subject of recent interest, at present there is no evidence to suggest that the effects of NO will be different in innervated kidneys.

Although SNAP elicited renal vasodilation, autoregulatory efficiency of RBF and GFR remained intact during SNAP administration. There was a significant leftward shift toward control in the slope of the linear portion of the pressure-flow curve during SNAP infusion in NLA-treated dogs. These findings further support our earlier conclusions[8,9] that NO primarily influences an autoregulatory-independent component of RVR. Consistent with the findings during inhibition of

endogenous NO production in anesthetized dogs, exogenous NO administration in these experiments also failed to elicit changes in GFR. These findings are consistent with the concept that NO exerts a proportionate influence on both preglomerular and postglomerular resistance segments.[28]

The results of this study demonstrate that increases in NO levels in the kidney induced by the administration of SNAP exert diuretic and natriuretic actions. The natriuretic response occurred in the absence of changes in filtered sodium load, which further supports the hypothesis that NO exerts an inhibitory influence on tubular sodium reabsorptive processes in the kidney.[9,11] However, these data do not provide further clarification of the mechanism involved in NO-induced changes in tubular reabsorptive function, which may be due to a direct inhibitory action of NO on epithelial sodium transport[29] or may occur as a consequence of an altered intrarenal hemodynamic environment.[30-32] Further studies are required to elucidate the exact mechanism and the nephron segments responsible for NO-induced inhibition of tubular sodium reabsorption.

As reported earlier,[9] NLA administration in anesthetized sodium-replete dogs in this study resulted in marked attenuation of the urine flow and sodium excretory responses to reductions in RAP. Although there was a partial restoration in the magnitude of $U_{Na}V$ after addition of SNAP, the excretory responses to reductions in RAP remained attenuated during SNAP administration. In these experiments, intrarenal SNAP infusion restored nearly 50% of the $U_{Na}V$ rate inhibited during NLA administration. This finding demonstrates that the marked attenuation in the slope of the pressure-natriuresis relation during NO inhibition was not due to the effects of NLA to lower basal sodium excretion, which could limit further decreases in sodium excretory values during reductions in RAP. The lack of a complete reversal of the responses to NLA during SNAP infusion may be due to an inadequate dose of SNAP used in this study to replace the basal production of NO in the kidney inhibited by NLA. However, higher doses of SNAP were not used in this study to avoid large decreases in systemic pressure. This is an obvious problem in studies involving intra-arterial infusion, which leads to substrate overflow and loss of agent to the circulation.

A constant infusion of exogenous NO in the renal artery in these NLA-treated dogs failed to increase the slopes of the relations between arterial pressure and $U_{Na}V$ or FE_{Na}. These interesting findings suggest that a simple absence of NO in the NLA-treated dogs was not the cause of attenuation in the pressure-induced natriuretic responses. The replenishment of NO in these NLA-treated dogs reversed $U_{Na}V$ toward control levels, yet the pressure-natriuresis curve remained attenuated, as during NLA infusion alone. This pattern is clearly different from those previously observed in response to other vasodilator or natriuretic agents that have usually caused a marked augmentation of the slope of the pressure-natriuresis relation.[9,22,33] This suggests a more direct association between NO and the mechanism responsible for pressure natriuresis.

From the present study we cannot determine the amount of NO delivered intrarenally by the administered dose of SNAP or the actual intrarenal NO levels. Considering the magnitude of the sodium excretory responses to changes in RAP, it may be assumed that the intrarenal NO levels achieved were similar to those present in the kidney at RAP levels of approximately 100 mm Hg before NO blockade. These results indicate that the amounts of endogenous NO present in the kidney at a RAP of 125 or 150 mm Hg during the control period were more than the amount of NO delivered by the SNAP infusion. This suggests that increases in RAP under control conditions increases endogenous NO production in the kidney. Thus, these data support the hypothesis that an alteration in the formation and release of NO by endothelial cells occurs in response to changes in RAP and may be an essential component of the mechanism responsible for pressure-induced diuretic and natriuretic responses in the kidney.[9] Further studies will be required to quantitate the intrarenal NO activity at different levels of arterial pressure and to examine the causal relation between such NO activity and observed changes in $U_{Na}V$.

In conclusion, the results of the present investigation demonstrate specific actions of NO to elicit renal vasodilation and natriuresis. Furthermore, these data suggest that alterations in intrarenal NO activity during changes in arterial pressure are requisite for full expression of pressure-natriuretic responses.

Acknowledgments

This work was supported by grant HL-18426 from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Md, and by a Young Investigator Award from the National Kidney Foundation, New York, NY. We thank George Prophet and Anka Hymel for excellent technical assistance and Agnes C. Buffone for preparing the manuscript. We are grateful to Dr Louis J. Ignarro, Department of Pharmacology, University of California, Los Angeles, for the SNAP used in this study.

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Hypertension. 1993;22:535-541
doi: 10.1161/01.HYP.22.4.535
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0194-911X. Online ISSN: 1524-4563