Nitric Oxide Increases Renal Blood Flow by Interacting With the Sympathetic Nervous System

Kazuhiro Kumagai, Hiromichi Suzuki, Masashi Ichikawa, Masahito Jimbo, Marohto Murakami, Munekazu Ryuzuki, Takao Saruta

Abstract To investigate whether changes in renal blood flow induced by nondepressor doses of l-arginine, the precursor of nitric oxide, are mediated by a sympathetic neural mechanism, we examined the following in conscious rabbits: (1) the effects of intravenous infusion of l- or d-arginine (15 to 200 μmol/kg per minute) on renal blood flow and renal sympathetic nerve activity with or without intravenous infusion of a nonpressor dose of Nω-monomethyl-l-arginine (L-NMMA), a nitric oxide synthase inhibitor, and (2) the effects of L-arginine on renal blood flow after renal denervation with or without L-NMMA pretreatment. In renal innervated rabbits, L-arginine (100 and 200 μmol/kg per minute) increased renal blood flow by 9±2 and 16±3 mL/min (P<.05, respectively) and decreased renal sympathetic nerve activity by 12±4% and 19±3% of control (P<.05, respectively). In contrast, no changes occurred in any variable during d-arginine infusion. L-NMMA attenuated the renal blood flow and renal sympathetic nerve activity responses to L-arginine (P<.05). In renal denervated rabbits, L-NMMA also attenuated the renal blood flow responses to L-arginine (P<.05) and abolished them (P<.05) compared with those in renal innervated rabbits. All renal blood flow responses to L-arginine were accompanied by parallel changes in plasma l-citrulline concentration. These results indicate that exogenous L-arginine decreases renal sympathetic nerve activity and that the reduction in renal sympathetic nerve activity could contribute to the increase in renal blood flow as well as other actions of nitric oxide, such as a direct vasodilator action on vascular smooth muscle tone, suppression of adrenergic nerve transmission in the blood vessels, and inhibition of the release of vasoactive substances. (Hypertension. 1994;24:220-226.)

Key Words • endothelium-derived relaxing factor • nitric oxide • arginine • renal circulation • sympathetic nervous system

Endothelium-derived relaxing factor (EDRF) relaxes vascular smooth muscle. Nitric oxide (NO) is synthesized from endogenous l-arginine in various tissues and accounts for the biological actions of EDRF. Accumulating evidence has revealed that EDRF/NO plays an important role in regulating renal blood flow (RBF). Possible mechanisms such as a direct action on vascular smooth muscle tone, suppression of adrenergic nerve transmission in the blood vessels, and inhibition of the release of vasoactive substances have been proposed for the NO-mediated modulation of RBF. There are also potential interactions among these regulatory mechanisms. For example, recent studies in anesthetized animals suggest that there is an interaction between sympathetic nerve activity and NO. Lacolley et al have suggested that normal sympathetic discharge plays an important role in modulating the synthesis or release of vascular NO. It has also been reported that NO has a role in the central regulation of sympathetic tone. However, it is known that anesthesia alters sympathetic nerve activity and has effects on responses to NO synthesis inhibitors, so the results of the studies obtained with the use of anesthesia should be interpreted with caution.

Although there is evidence that exogenous L-arginine produces a vasodilator action by increasing NO production, to our knowledge there is no direct evidence that the effect of exogenous L-arginine on renal sympathetic nerve activity (RSNA) contributes to the modulation of RBF in conscious animals. The aim of the present study was to determine whether a sympathetic neural mechanism contributes to changes in RBF elicited by nondepressor doses of L-arginine in conscious rabbits. Therefore, we investigated the RBF and RSNA responses to nondepressor doses of L-arginine with or without pretreatment with Nω-monomethyl-L-arginine (L-NMMA), an NO synthase inhibitor, in renal innervated rabbits. In addition, since we hypothesized that the inhibitory action of NO on RSNA would contribute to the RBF response to L-arginine, we investigated whether an absence of a reduction in RSNA, i.e., renal denervation, alters the RBF responses to L-arginine with or without L-NMMA pretreatment.

Methods

General Procedures

Experiments were conducted in female Japanese White rabbits (Sankyo Laboratory Co) weighing 2.8 to 3.3 kg. All surgical and experimental procedures followed institutional animal care guidelines. Rabbits were housed singly in cages in a room with a constant temperature and 12-hour light/dark cycle and were fed a commercial diet (Oriental Yeast Co) and water ad libitum.

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Surgical Procedures

For all surgical preparations, rabbits were anesthetized with pentobarbital sodium (30 mg/kg IV, Abbott Laboratories). The surgery for catheterization, implantation of the RBF probe and renal nerve electrodes, and renal denervation were performed under sterile conditions in accordance with the guidelines for animal welfare. After all surgical procedures, ampicillin (10 mg/d IV) diluted with 0.9% sterile saline was given.

Arterial and Venous Catheterization

The right external jugular vein was catheterized with two polyethylene catheters (PE-60, Becton Dickinson, Clay Adams) with silicone elastomer tubing, and the left subclavian artery was also catheterized. All catheters were passed subcutaneously to the back of the neck and fixed. All catheters were protected with a custom-made stockinette jacket. To avoid clotting, each catheter was flushed with heparin (1000 U/mL) in 0.9% sterile saline every day.

RBF Probe Implantation and/or Renal Denervation

In renal innervated rabbits, the right kidney was exposed retroperitoneally. The renal artery and renal nerve were identified and carefully dissected from the renal vein with a dissecting microscope. An electromagnetic flow probe (FH, Nihon Kohden Co) was fixed on the right renal artery close to its origin and carefully secured to the surrounding tissue and muscles. The flank incision was closed and the probe exteriorized at the back of the neck. The probe was protected with a custom-made stockinette jacket. In another group of rabbits, the right or left kidney was exposed retroperitoneally. The denervation procedure was performed according to a previous study. With a dissecting microscope, the renal artery was stripped of its adventitia and coated with a solution of 10% phenol in ethanol during a 30-minute period. During the application of phenol, the kidney and adjacent tissues were carefully protected from exposure to the solution. Rabbits showing spasm of the renal artery were discarded. The same procedure was applied to the other side, and the flank incision was closed. At least 8 days after renal denervation, an electromagnetic flow probe was placed on the right renal artery as described above.

Renal Nerve Electrode Implantation

In renal innervated rabbits, at least 7 days after flow probe implantation, RSNA recording techniques were used to obtain multifiber recordings of postganglionic RSNA as previously described. With rabbits in a shielded cage, the left kidney was exposed via a retroperitoneal approach through a left flank incision. The flank incision was closed. At least 8 days after renal denervation, an electromagnetic flow probe was placed on the right renal artery as described above.

Data Analysis

The arterial catheter was connected to a transducer (TP-200T, Nihon Kohden) for measurement of arterial pressure (AP-611G, Nihon Kohden) and heart rate (HR) (AT-601G, Nihon Kohden). The RSNA recording electrodes were connected to a high-impedance probe (JB101J, Nihon Kohden), which was connected to a differential amplifier (AVB-10, Nihon Kohden) with a band-pass filter (low, 50 Hz; high, 3 kHz). The amplified (×10 000 to ×20 000) and filtered activity was monitored on an oscilloscope (VC-10, Nihon Kohden). In accordance with previous reports, the root mean square (RMS) of RSNA was defined as the whole-nerve activity obtained by rectifying and integrating activity with an RMS integrator (E-601G, Nihon Kohden) that had a time constant of 28 milliseconds, and the mean RSNA was defined as the RMS of RSNA further filtered at 0.08 Hz for quantification. Background noise was determined when nerve activity was eliminated by increasing arterial pressure with an infusion of phenylephrine (40 μg/kg per minute). This value was subtracted from all the experimental values of RSNA. The initial baseline value of integrated mean RSNA was defined as 100%. The flow probe was connected to a flowmeter (MFV-1200, Nihon Kohden). Arterial pressure, mean arterial pressure (MAP), HR, original renal neurogram, mean RSNA, and RMS of RSNA were recorded on a thermal array recorder (RTA-1300, Nihon Kohden). RBF was recorded on a multichannel polygraph (RM-6000, Nihon Kohden).

Blood Collection and Analysis

Blood samples for measurement of plasma L-citrulline concentration were centrifuged at 4°C. Plasma for measurement of L-citrulline concentration was stored at −80°C until assay. As previously described, plasma L-citrulline concentration was determined with an automated amino acid analyzer (JLC-300V, JEOL Co).

Experimental Protocols

Experiment 1

In this experiment, we investigated the effects of nonpressor doses of L-arginine on HR, RBF, and RSNA in renal innervated rabbits (n=11). A minimum of 2 days after renal nerve electrode implantation, conscious rabbits were placed unrestrained in a stainless steel cage. L-Arginine (n=6) or D-arginine (n=5) hydrochloride (Sigma Chemical Co) was infused intravenously. The concentration of each solution was 100 mg/mL, and the pH was 5 to 6. A randomized design was used to choose L- or D-arginine and their doses. Sixty minutes were allowed for equilibration of MAP, HR, and RSNA. After the first 20-minute pretreatment period, infusion of L- or D-arginine (15 to 200 μmol/kg per minute IV) was performed over four consecutive 20-minute periods.

Experiment 2

In this experiment, we examined whether pretreatment with a nonpressor dose of L-NMMA abolished the RBF, HR, and RSNA responses to nonpressor doses of L-arginine in renal innervated rabbits (n=5). We used a nonpressor dose of L-NMMA to avoid the changes in HR and RSNA that are normally elicited by an increase in arterial blood pressure. In renal innervated rabbits, 60 minutes were allowed for equilibration of MAP, HR, and RSNA. Forty minutes after initiation of a constant infusion of 0.25 μmol/kg per minute IV L-NMMA, infusion of L-arginine (15 to 200 μmol/kg per minute IV) was superimposed.

Experiment 3

In this experiment, we hypothesized that the inhibitory action of NO on RSNA could contribute to the RBF response to L-arginine, we investigated whether the absence of a reduction in RSNA, ie, renal denervation, alters the RBF responses to nonpressor doses of L-arginine (n=4). In renal innervated rabbits, L-arginine was infused intravenously. Sixty minutes were allowed for equilibration of MAP and HR. After the first 20-minute pretreatment period, L-arginine (15 to 200 μmol/kg per minute IV) was infused.
Experiment 4

In this experiment, we examined whether pretreatment with a nonpressor dose of L-NMMA abolished the RBF responses to nondepressor doses of L-arginine in renal denervated rabbits (n=4). In renal denervated rabbits, L-NMMA was infused alone and simultaneously with L-arginine. Sixty minutes were allowed for equilibration of MAP and HR. Forty minutes after initiation of a constant infusion of 0.25 μmol/kg per minute IV L-NMMA, an infusion of L-arginine (15 to 200 μmol/kg per minute) was superimposed.

Experiment 5

In this experiment, to confirm whether exogenous nonpressor doses of L-arginine stimulate the intrinsic pathway from L-arginine to NO, we measured plasma concentrations of L-citrulline, a product of the L-arginine–NO pathway. A blood sample (3.5 mL) was obtained from renal innervated rabbits (n=5) at the end of a pretreatment period with L- or D-arginine chosen by a randomized design and at each arginine dose (15 to 200 μmol/kg per minute). In the same rabbits, after at least a 5-day interval, we administered the other arginine and obtained blood samples similarly. In other renal innervated rabbits (n=4) with L-NMMA pretreatment (0.25 μmol/kg per minute), a blood sample was obtained at the end of the pretreatment period with L-arginine and each dose of L-arginine. In other renal denervated rabbits (n=5), a blood sample was obtained at the end of the pretreatment period with L-arginine and each dose of L-arginine. In the same rabbits, after at least a 5-day interval, with L-NMMA pretreatment, a blood sample was obtained at the end of the pretreatment period with L-arginine and each dose of L-arginine. Each blood sample was replaced by the same volume of blood from a donor rabbit.

Statistical Analysis

Results are expressed as mean±SEM. Multiple comparisons were made using two-way ANOVA for repeated measures and the Newman-Keuls or Dunnett’s test. Changes were considered statistically significant at a value of P<.05.

Experiment 1

As shown in Fig 1 and Table 1, in the renal innervated rabbits L-arginine (15 to 200 μmol/kg per minute) did not alter MAP. Only at the highest doses administered (100 to 200 μmol/kg per minute) did L-arginine significantly increase HR by 6% to 8% (P<.05), increase RBF by 23% to 40% (P<.05), and reciprocally decrease RSNA by 12% to 19% (P<.05) compared with preinfusion values and corresponding D-arginine infusion values. In contrast, D-arginine caused no significant changes in MAP, HR, RBF, or RSNA compared with preinfusion values.

Experiment 2

As shown in Fig 2 and Table 2, in the renal innervated rabbits L-NMMA pretreatment did not alter MAP, HR, RBF, or RSNA. With a constant infusion of L-NMMA, in contrast to experiment 1, L-arginine (100 μmol/kg per minute) did not alter HR, RBF, or RSNA, and only at the highest dose administered (200 μmol/kg per minute) did it significantly increase HR by 7% (P<.05), increase RBF by 21% (P<.05), and concurrently decrease RSNA by 12% (P<.05) compared with preinfusion values. Compared with conditions without L-NMMA treatment, the highest L-NMMA dose showed an increase in RBF of 21% versus 40% (Fig 3) and decrease in RSNA of 12% versus 19%. The HR response to L-arginine was essentially the same either with or without L-NMMA treatment.

Experiment 3

As shown in Table 1, in the renal denervated rabbits L-arginine (15 to 100 μmol/kg per minute) did not alter MAP, HR, or RBF, and only at the highest dose administered (200 μmol/kg per minute) did it elicit a small increase in HR of 3% (P<.05) and a significant

Results

Fig 1. Line graphs show mean arterial pressure (MAP), heart rate (HR), renal blood flow (RBF), and renal sympathetic nerve activity (RSNA) responses to L-arginine (●, n=6) and D-arginine (○, n=5) in renal innervated rabbits. Values are mean±SEM. *P<.05 compared with preinfusion value; †P<.05 compared with corresponding D-arginine infusion value.
TABLE 1. Effects of Arginine In Renal Innervated and Denervated Rabbits

<table>
<thead>
<tr>
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<td>RSNA, % of control</td>
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MAP indicates mean arterial pressure; INN, innervated; L-Arg, L-arginine; D-Arg, D-arginine; DNX, denervated; HR, heart rate; RBF, renal blood flow; and RSNA, renal sympathetic nerve activity. Values are mean±SEM; n=6 in INN/L-Arg, n=5 in INN/D-Arg, and n=4 in DNX/L-Arg.

*P<.05 compared with preinfusion value.
†P<.05 compared with corresponding D-Arg infusion value.
‡P<.05 compared with corresponding INN.

increase in RBF of 18% (P<.05) compared with preinfusion values. Compared with experiment 1, the highest dose showed an increase in RBF of 18% versus 40% (Fig 3) and increase in HR of 3% versus 8%.

Experiment 4

As shown in Fig 2 and Table 2, in the renal denervated rabbits L-NMMA pretreatment did not affect MAP, HR, or RBF. With a constant infusion of
TABLE 2. Effects of L-Arginine With N\textsuperscript{\textdegree}-Monomethyl-L-Arginine Pretreatment In Renal Innervated and Denervated Rabbits

<table>
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<tr>
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MAP, mm Hg

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HR, bpm

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RBF, mL/min

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RSNA, % of control

| Group | 100    | 100±3 | 100±4 | 100±3 | 98±3  | 97±4  | 88±4* |

Definitions are as in Table 1; and L-NMMA, \textsuperscript{\textdegree}O-monomethyl-L-arginine. Values are mean±SEM; n=4 in INN, n=4 in DNX.

*P<.05 compared with preinfusion value.
†P<.05 compared with corresponding DNX.

L-NMMA, even the highest dose of L-arginine administered (200 \textmu mol/kg per minute) did not alter HR or RBF compared with preinfusion values. Compared with experiment 3, the only difference in the data was the RBF response, which showed a smaller increase of 3% versus 18% (Fig 3). The HR response to L-arginine was essentially the same either with or without L-NMMA treatment. Compared with experiment 2, RBF increased 3% versus 21% (Fig 3), and HR increased 3% versus 7%. With or without renal denervation, L-NMMA treatment elicited decreases in the RBF response to L-arginine almost equivalently (from 40% to 21% increase versus from 18% to 3% increase) and did not alter the HR response (from 8% to 7% increase versus from 3% to 3% increase).

**Experiment 5**

Fig 4 summarizes the changes in plasma L-citrulline concentration. In the renal innervated rabbits the highest L-arginine doses administered (100 and 200 \textmu mol/kg per minute) increased plasma L-citrulline concentration from 78±15 to 118±14 and 141±17 nmol/mL (P<.05, respectively). In contrast, \textsuperscript{\textdegree}arginine had no effect on plasma L-citrulline concentration. In the renal innervated rabbits even with L-NMMA pretreatment the highest L-arginine dose administered (200 \textmu mol/kg per minute) increased plasma L-citrulline concentration from 76±8 to 124±13 nmol/mL (P<.05). In the renal denervated rabbits L-arginine at only the highest dose
increased plasma l-citrulline concentration from $66 \pm 11$ to $116 \pm 11$ mmol/mL ($P<0.05$). In the renal denervated rabbits with L-NMMA pretreatment even at the highest dose l-arginine did not alter plasma l-citrulline concentration.

Discussion

The present data indicate that infusion of the NO precursor l-arginine (100 to 200 $\mu$mol/kg per minute) results in formation of NO in an amount that does not alter arterial pressure but increases RBF. This is supported by the findings that the RBF responses to l-arginine were mirrored by increases in l-citrulline concentration, that d-arginine did not have such effects, and that a nonpressor dose of the NO synthase inhibitor L-NMMA attenuated the RBF responses to l-arginine. Therefore, our findings support the concept that exogenous l-arginine produces increases in RBF, at least in part mediated by increasing NO formation, and also suggest that the renal circulation is especially sensitive to NO formation.

The present findings that the l-arginine–induced increases in RBF were accompanied by reciprocal changes in RSNA and that these responses were attenuated by a nonpressor dose of L-NMMA support the hypothesis that l-arginine–induced NO formation has an inhibitory action on RSNA. Several in vitro studies have shown that endothelium-derived vasoactive factors modulate adrenergic neurotransmission in the blood vessels. Tesfamariam et al.\(^{21}\) suggested that spontaneously released EDRF as well as disposition of norepinephrine in the endothelial cells contributes to the inhibition of contraction caused by electrical stimulation. Greenberg et al.\(^{14}\) suggested that the endothelium, in part through EDRF, could inhibit norepinephrine release elicited by transmural nerve stimulation. In vivo studies\(^{12,22}\) have provided evidence that L-NMMA increases RSNA in anesthetized animals, suggesting that NO inhibits RSNA. Thus, our findings support the hypothesis that l-arginine–induced NO formation has an inhibitory effect of NO on RSNA are in accordance with recent studies.

Although the present data suggest an inhibitory effect of NO on RSNA, we did not observe that L-NMMA treatment increased RSNA. Major differences between recent studies\(^{12,22}\) and ours could be responsible. First, we performed the present experiments with rabbits in the conscious state, because anesthesia is known to produce several untoward effects on sympathetic neural regulation\(^{13}\) and on responses to NO synthase inhibitors.\(^{14}\) Second, the L-NMMA dose we used was a nonpressor dose and much smaller than the doses used in studies by other investigators.

In the renal innervated rabbits the extent of the L-NMMA–induced attenuation of the RBF response to l-arginine seemed inconsistent with the extent of the decreases in RSNA. This discrepancy could have resulted from the presentation of RSNA data as percent changes. Since we measured the whole-nerve activity, we cannot compare the difference in 100% of control as well as baseline of actual RSNA between control and L-NMMA–treated rabbits. It is possible that L-NMMA increased baseline RSNA, and consequently, data presented as percent change might be very different if experiments started at a different actual level of nerve activity.

It has been suggested that normal sympathetic discharge could enhance basal synthesis or release of vascular NO,\(^{11}\) so we hypothesized that an absence of reduction in RSNA, ie, renal denervation, elicits changes in the RBF response to l-arginine. We found that renal denervation attenuated the RBF response to l-arginine without affecting the basal level of RBF. This could indicate that renal denervation decreases basal synthesis or release of vascular NO without affecting the basal level of RBF and as a result elicits a smaller increase in the RBF response to l-arginine. Additionally, it is conceivable that possible attenuation of the synthesis or release of vascular NO resulted from changes in the interaction of the vasculature and NO by renal denervation.

The observation that a nonpressor dose of L-NMMA had no effect on the basal level of RBF with or without renal denervation suggests that L-NMMA has little or no effect on the basal synthesis or release of vascular NO. In addition, the finding that L-NMMA suppressed the RBF responses to l-arginine almost equivalently with or without renal denervation demonstrates that it inhibits l-arginine–induced NO formation. Taken together, these data seem to show that renal denervation could decrease basal synthesis or release of vascular NO, whereas a nonpressor dose of L-NMMA inhibits the RBF responses to l-arginine, and that consequently both renal denervation and L-NMMA abolish the RBF responses to l-arginine. This may imply that the basal synthesis or release of vascular NO could be linked to exogenous l-arginine–induced NO. Alternatively, the data suggest that l-arginine–induced renal NO has two separate and seemingly additive effects on renal hemodynamics: a direct NO-mediated vasodilation and an inhibition of renal vascular tone by inhibition of RSNA.

The observations that the increase in the plasma concentration of l-citrulline with 100 $\mu$mol/kg per minute l-arginine was blocked by renal denervation and that L-NMMA did not block the increase in l-citrulline with 200 $\mu$mol/kg per minute l-arginine in the renal innervated state seem complicated. Both observations could be explained in part by the potential mechanism of renal denervation, ie, lack of normal sympathetic discharge, attenuating the synthesis or release of vascular NO, since Lacolley et al.\(^{11}\) have suggested that normal sympathetic discharge enhances the synthesis or release of vascular NO and that neurogenically derived vasoconstriction enhances NO generation. Therefore, the changes in plasma l-citrulline concentration reflect the fact that NO interacts with RSNA: on one hand, sympathetic discharge could enhance the synthesis or release of vascular NO, whereas on the other, NO could inhibit sympathetic discharge and presynaptic norepinephrine release.

In conclusion, our present findings indicate that exogenous l-arginine elicits a reduction in RSNA and that the reduction in RSNA could contribute to the increase in RBF as well as other mechanisms such as a direct vasodilator action of NO on vascular smooth muscle tone, suppression of adrenergic nerve transmission in the blood vessels, and inhibition of the release of vasoactive substances.

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