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Effect of Nitric Oxide Synthase Inhibition on Intrarenal Oxygenation as Evaluated by Blood Oxygenation Level-Dependent Magnetic Resonance Imaging

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Abstract

Objective—To investigate the feasibility of studying renal effects of nitric oxide synthase inhibition (NOSi) in humans by blood oxygenation level-dependent (BOLD) MRI. Nitric oxide (NO) is known to play a key role in the pathophysiology of hypertension and previous reports suggest reduced bio-availability of NO in the kidneys of hypertensive rats and hence show reduced response to NOSi using BOLD MRI. Ability to perform similar studies in humans could potentially lead to detection of early changes before development of symptoms, and to monitor novel interventions targeted toward improved NO bioavailability.

The specific goals for this study were: (1) to examine whether lower doses and dose rate of administration of NOSi such as those previously used in humans can be detected by BOLD MRI in rat kidneys, (2) to compare changes in R_2^* to direct measures of renal medullary oxygen levels and blood flow using invasive probes (OxyLite/OxyFlo), and (3) to examine for the first time the effect of NOSi on intrarenal oxygenation in humans.

Material and Methods—In rat kidneys, acute changes in renal tissue oxygenation induced by different doses (2, 4, and 10 mg/kg) of N-nitro-L-arginine methyl ester were studied in 36 Sprague Dawley rats, which were equally divided into BOLD MRI and OxyLite/OxyFlo groups. Similarly in humans, acute changes in renal oxygenation were induced by 2 different NOS inhibitors N^G-monomethyl-L-arginine (4.25 mg/kg) in 7 volunteers and N-nitro-L-arginine methyl ester (2 mg/kg and 4 mg/kg) in 6 healthy young volunteers.

A multiple gradient echo sequence was used in both rats (TE = 4.4–57.8 milliseconds with 3.6 milliseconds interecho spacing) and humans (TE = 6.4–40.8 milliseconds with a 2.3 milliseconds interecho spacing) to acquire 16 T_2^* -weighted images. R_2^* maps were constructed by fitting a single exponential decay to the image data on pixel by pixel basis. R_2^* measurements in the cortex and medulla were performed by regions of interest analysis. Measurements were performed before and during infusion of NOSi.

Results—In rats, NOSi decreased medullary pO_2 and blood flow in a dose-dependent manner, and BOLD MRI showed an increase in medullary R_2^* consistent with the invasive pO_2 measurements. In humans, BOLD MRI similarly showed an increase in medullary and cortical R_2^* after NOSi in a dose-dependent manner. In both rats and humans, the R_2^* values fell back toward baseline before the end of the infusion period.

Conclusion—Comparison of BOLD MRI measurements with those using invasive probes suggests that changes in blood flow are at least partly responsible for observed changes with BOLD MRI. Monitoring changes after NOSi by renal BOLD MRI in vivo in human kidneys are feasible, and preliminary findings are consistent with observations in rat kidneys. Future studies are warranted to fully understand the apparent reversal in R_2^* changes during the infusion of NOSi.

Keywords

hypertension; kidney; nitric oxide; oxygenation; MRI

A number of recent studies have provided improved understanding of the pathobiology behind development of hypertension^{1–3} and a role for nitric oxide (NO) has been identified.^{4–10} All these are based on animal models using invasive probe measurements and findings cannot be directly translated to humans because of the lack of noninvasive methods. With the developments in in vivo imaging, it is now possible to evaluate responses to vasoactive agents in human subjects. Brachial artery ultrasound studies have been used to show that subjects at risk for development of hypertension and vascular disease exhibit reduced hyperemic responses (known to be NO dependent) compared with controls.¹¹

Blood oxygenation level-dependent (BOLD) magnetic resonance imaging (MRI) has been shown to be useful in evaluating intrarenal oxygenation noninvasively both in humans and animal models.^{12–19} The technique is particularly suited for studies of the renal medulla because it is naturally hypoxic and falls on the linear portion of the hemoglobin oxygen desaturation curve, where the technique is most sensitive.²⁰ Effects of prostaglandin^{21,22} and nitric oxide synthase (NOS)²³ inhibition have been studied previously using BOLD MRI. Acute increase in renal medullary hypoxia has been reported after a bolus administration of 10 mg/kg N^G -nitro-L-arginine methyl ester (L-NAME) in rats.²³ In a subsequent study, it was shown that such a response to L-NAME was absent in the kidneys of hypertensive rats,²⁴ consistent with known lack of nitric oxide availability.²⁵ Our overall objective is to extend these findings to humans. Before attempting such a study, we wanted to prove feasibility of performing similar measurements in humans. Although considered to be investigational, nitric oxide synthase inhibitors (NOSi) have been used safely in humans including patients. However, they were administered at lower doses and dose rates compared with animal studies.^{26–32} Two specific NOS inhibitors, N^G -mono-methyl-L-arginine (L-NMMA) and L-NAME have been used in humans.²⁹ Owing to the fact that L-NMMA is endogenous to humans, its use has been preferred. However, the efficacy of L-NMMA in terms of increased blood pressure response has been shown to be much less compared with L-NAME.^{29,33} Therefore, in this preliminary study, we studied the effects of both L-NMMA and L-NAME. Two dose rates of L-NAME (2 mg/kg and 4 mg/kg) were studied based on the reports to date.²⁹

This study was designed specifically to test feasibility of using BOLD MRI to monitor effects of NOSi in humans. Because the dose and dose rates of NOSi used in humans is relatively low compared with animal studies to date (2 vs. 10 mg/kg, infusion over 30 minutes vs. bolus administration), in this study, we wanted to first study the dose response in rat kidneys when administered as an infusion over 30 minutes. We also wanted to acquire correlative pO₂ and blood flow measurements to aid future interpretation of BOLD MRI responses observed after NOSi. We then wanted to make similar measurements using BOLD MRI in humans.

MATERIALS AND METHODS

Rats

All animal experiments were performed in accordance with the applicable animal welfare regulations and with the approval of the Institutional Animal Care and Use Committee. Male Sprague-Dawley (SD) rats (weighing 250–350 g, age 8–10 weeks) were obtained from Harlan Bioproducts for Science (Indianapolis, IN) and housed at 25°C with 12 hours light/dark cycle and free access to food and water. Experiments were performed in a total of 36 SD rats, which were equally divided into BOLD MRI and OxyLite groups. These 2 groups were further subdivided by dose (2, 4, or 10 mg/kg) of L-NAME (6 each).

OxyLite Group

The combined OxyLite/OxyFlo probe allowed for simultaneous acquisition of pO₂ and blood flow within a couple of 100 microns of one another. OxyLite measures pO₂ using the fluorescence lifetime of a chromophore fixed to the tip of an optical fiber that is inserted into the tissue.³⁴ Oxyflo is a Doppler probe that measures blood flow in arbitrary units. The probes used in this study were combined probes with a tip size ~450 μm (OxyLite with temperature probe ~350 μm + OxyFlo ~ 100 μm). The probes are calibrated at the manufacturer before shipment; the calibration for each probe was scanned into the computer by using a barcode wand. The pO₂ and blood flow signals from the probes were a 5-second average value and were recorded to disk with the use of a data-acquisition system (Powerlab, Chart v5 for Windows—AD Instruments, Colorado Springs, CO).

Rats were anesthetized with Ketamine (60–100 mg/kg ip, Abbott Laboratories, North Chicago, IL) and thiobutabarbital (Inactin, 100 mg/kg IP, St. Louis, MO). Catheters were placed in the trachea (PE-250), femoral artery, and vein (PE-50). Bovine serum albumin (BSA, 4 mg/dL) in saline was infused throughout the experiment (2 mL/h), and mean arterial blood pressure (MAP) was continuously monitored by a pressure transducer system (Kent Scientific, Litchfield, CT) connected to the arterial line before and during the infusion of L-NAME (Sigma-Aldrich Corp., St. Louis, MO). The rat core temperatures were monitored and maintained at approximately 37°C with a heating table (Harvard Apparatus, Holliston, MA). The urinary bladder was incised to prevent urinary retention. The left kidney was exposed through a midline incision and mechanically fixed. OxyLite in conjunction with Oxyflo (Oxford Optronix, Oxford, UK) were used to simultaneously and continuously monitor tissue blood flow, oxygenation, and temperature at the same location. The probes were attached to a micromanipulator and inserted into a depth of 3.0 to 4.0

mm^{35,36} to determine the changes in outer medullary pO₂ and blood flow. Positioning of the probes was confirmed at the end of the experiments by dissection similar to our previous report.³⁷

Baseline readings were recorded for 15 minutes, after which L-NAME infusion was initiated. L-NAME (2 mg/kg, 4 mg/kg or 10 mg/kg) was infused over 30 minutes using a pump (Genie Plus, Kent Scientific, Litchfield, CT) connected to the i.v. line. Data were collected continuously at a sampling frequency of 20 Hz before, during, and after L-NAME infusion. Data collection is described in the following timing diagram.

BOLD MRI Group

After anesthesia, catheters were placed in the femoral artery and vein (PE-50). MAP was continuously monitored by a pressure transducer system connected to the arterial line. MRI studies were performed on a short bore Signa Twin speed 3.0 T (GE Healthcare, Milwaukee, WI) using a multiple gradient echo sequence (TR/Flip angle/FOV/BW/matrix/Thk/NXE = 70 milliseconds/30-degree/10 cm/42 k Hz/256 × 256/2 mm/10) to acquire 16 T₂*-weighted images (TE is 4.4 –57.8 milliseconds with 3.6 milliseconds interecho spacing). A standard commercial quadrature transmit-receive extremity coil was used for signal reception. After obtaining a set of baseline images, L-NAME (2 mg/kg, 4 mg/kg, or 10 mg/kg) was infused over 30 minutes. BOLD MRI measurements were obtained every 3 minutes. Data collection is described in the following timing diagram.

Human

One female and 6 male healthy volunteers (age: 27.6 ± 6.0 ; weight: 88.3 ± 15.6 kg) participated in the L-NMMA study. Six additional male healthy volunteers (age: 28.8 ± 6.1 ; weight: 84.2 ± 10.9 kg) participated in the L-NAME study with 2 different doses on 2 different days at least 1 week apart. After being introduced to the research goals and procedures, each volunteer gave informed consent in a protocol approved by our Institutional Review Board. The subjects came to the study after abstaining from food and water for about 12 hours overnight.

Experiments were conducted on a GE Signa 3.0T whole body scanner (GE Healthcare, Milwaukee, WI), using a standard 4-element or 8-element torso phased array coil for signal reception. BOLD imaging was performed using a multiple gradient echo (mGRE) sequence

with a water-selective excitation pulse (TR/FA/BW/FOV/matrix = 60/30/62.5 kHz/36 × 27/256 × 256). Sixteen T₂*-weighted images were obtained in each of 5 axial planes within a single breath-hold of about 12 seconds. TE varied from 6.4 to 40.8 milliseconds with a 2.3 milliseconds interecho spacing. A slice thickness of 5 mm was used.

MAP was calculated from systolic and diastolic pressures measured using an MRI compatible patient monitoring system (Magnitude, In vivo). After acquiring baseline data, NOS inhibitor (either L-NMMA or L-NAME) was infused intravenously. L-NMMA (Clinalfa, Bad Soden, Germany) was administered at a dose of 3 mg/kg over 6 minutes, followed by an infusion of 3 mg/kg/h over the remaining 24 minutes, making a total of 4.25 mg/kg.³⁸ The dose of L-NAME (Clinalfa) was 2 mg/kg or 4 mg/kg,²⁹ administered over 30 minutes followed by 15 minutes L-arginine (Clinalfa) infusion at dose of 200 mg/kg to reverse the NOSi effect.²⁹ BOLD and MAP data were acquired every 3 minutes for 30 minutes during the infusion of the NOS inhibitor. Data collection is described in the following timing diagram.

Image Analysis

R₂* maps were constructed using FUNCTOOL (GE Healthcare, Milwaukee, WI) by fitting a single exponential decay to the image data on pixel by pixel basis. The change in brightness in R₂* map reflects the change in oxygenation. To quantitatively express the change induced by the NOS inhibitor, circular regions of interest (ROI) covering at least 20 pixels were then drawn on the anatomic template and used as masks to extract average values from the R₂* maps by LPL. ROIs were defined in each of the 5 slices acquired (in humans), and over both kidneys, to obtain 10 to 20 measurements each for the cortex and medulla per time point. Each set of data were then combined to obtain a single mean value for each, cortex and medulla, per subject, per time point. In rats, data was acquired only at a single slice location and many times only in 1 kidney.

Statistical Analysis

Data are presented as mean ± SE. The statistical significance of differences between pre- and post-L-NAME was assessed using the 2-tailed paired Student *t*-test. A *P* < 0.05 was considered significant.

RESULTS

Rats

Figure 1 shows pre- and post-L-NAME R₂* maps with different L-NAME infusion doses from representative rats. The medulla in the post-L-NAME R₂* map is relatively brighter as compared with pre-L-NAME map for each dose, signifying a reduction in medullary

oxygenation. The R_2^* values in the medulla increased post- L -NAME with increasing doses. The window and level settings for pre- and post- L -NAME R_2^* maps were the same.

Table 1 summarizes measured baseline and peak values after L -NAME in MAP, pO_2 , and renal blood flow obtained from averaging data from all 6 animals in each group. Figure 2 illustrates the temporal changes of MAP, medullary R_2^* , pO_2 , and blood flow measurements during the 3 different doses of L -NAME infusion. Data are presented as a percent change compared with the baseline to accommodate data from different groups of animals on the same plot. All the 3 doses of L -NAME produced a dose-dependent increase in MAP (Fig. 2A) with a maximum change of 13.8%, 31.7%, 41.95% corresponding to 2, 4, 10 mg/kg L -NAME, respectively. With OxyLite/OxyFlo measurements, a dose-dependent reduction in pO_2 and blood flow in the renal medulla was observed during the 30 minutes infusion (Figs. 2C, D). The maximum decrease in pO_2 was 30.4%, 43.7%, 61.0%, and the maximum decrease in blood flow was 20.8%, 32.6%, 44.0%, corresponding to doses of 2, 4, 10 mg/kg of L -NAME. R_2^* similarly demonstrated a dose-dependent change. However, as seen in Figure 2B, values reach a maximum and then fall back toward the baseline values during the infusion period. Based on this observation, we have chosen to use the peak R_2^* value during the infusion as post-NOSi R_2^* measure. Figure 3 summarizes the individual pre- and post-NOSi measurements in the medulla.

Human

Representative R_2^* maps from 1 subject during L -NMMA infusion are shown in Figure 4. The medulla on both baseline and post- L -NMMA R_2^* map is brighter than the cortex because of its lower oxygenation status. During the infusion of L -NMMA, the medullary R_2^* increased compared with the baseline indicating a further reduction in oxygenation in this region.

Figure 5 displays a plot of MAP and R_2^* versus time during L -NMMA infusion. Medullary R_2^* reached the peak before showing a trend of falling back toward baseline levels. MAP also demonstrated a time varying response during NOSi infusion. We used peak values to represent post-NOSi R_2^* and MAP measures in Figures 6 and 7. We did not observe any correlation between the R_2^* and MAP temporal profiles, ie, the peak values for R_2^* and MAP did not necessarily coincide.

Pre- and post- R_2^* measurements in the renal medulla and cortex with L -NMMA and L -NAME are summarized in Figure 6. Similarly Figure 7 summarizes the MAP data. The peak R_2^* change ($R_2^* = \text{post-}R_2^* - \text{pre-}R_2^*$) in the medulla and cortex during L -NAME infusion in 4 mg/kg protocol is higher than that in 2 mg/kg protocol (Table 2). This is also true for peak MAP response (Table 2).

DISCUSSION

In rats, the results are consistent with previous reports⁸ and show a dose-dependent increase of MAP during L -NAME infusion. We also observed a dose-dependent change in R_2^* . Measurements with OxyLite/OxyFlo showed a dose-dependent decrease in renal medullary tissue pO_2 and blood flow. Because the tissue pO_2 measurements can be influenced by both

blood flow (oxygen supply) and oxygen consumption, these results indicate that blood flow changes during L-NAME infusion are at least partly responsible for the observed increase in renal medullary R_2^* .

It is important to note that no direct calibration of R_2^* against blood pO_2 measurements are feasible because BOLD MRI does not directly measure tissue pO_2 . However, relative trends after the administration of the pharmacologic agents can be compared. Although in principle OxyLite/OxyFlo measurements can be obtained simultaneously along with BOLD MRI, there are certain logistical hurdles to be cleared. The probes are MRI compatible, but appropriate probe holders need to be developed. Also, simultaneous measurements would mean that BOLD MRI measurements have to be made in externalized kidneys. In this preliminary study, we have only attempted to match trends observed with both techniques in different groups of animals.

Although there was a general agreement between the trends observed by BOLD MRI and invasive probe measurements, there was 1 striking difference observed during the infusion of L-NAME. In the OxyLite/OxyFlo study, pO_2 and blood flow changes were persistent over the duration of the study (Fig. 2), whereas with BOLD MRI, the change in R_2^* was relatively short lived and tended to return to the baseline before the completion of the L-NAME infusion. The effect was most pronounced at the highest dose.

The exact reason for this apparent discrepancy is not yet clear. A major difference between the BOLD MRI and invasive probe measurement is the fact that the animals were intact for the MRI acquisitions whereas the kidneys were exposed in the case of the OxyLite/OxyFlo measurements. It is also possible that insertion of the probes caused local injury and may not have been sensitive to the changes in the rest of the renal parenchyma. On the other hand, BOLD MRI measurements are sensitive to parameters other than just blood flow and oxygen consumption. The measurements are sensitive to blood volume changes and also regional hematocrit. Significant changes in blood volume may be associated with the administration of vasoactive agents especially over time.³⁹ The changes in blood volume could potentially lead to changes in R_2^* that are opposite in sign compared with those due to changes in blood flow and oxygenation. Their temporal responses may also be different from blood flow changes. These could potentially be responsible for the return to baseline that occurred during the infusion observed with BOLD MRI measurements.

In humans, preliminary data presented here demonstrates for the first time that R_2^* is a sensitive and effective indicator for monitoring renal effects of NOS inhibition. Both L-NMMA and L-NAME produced a significant increase in the medullary and cortical R_2^* although the magnitude of the change is larger in the medulla than the in cortex. Consistent with the MAP responses, the change in R_2^* was higher with 4 mg/kg L-NAME compared with 4.25 mg/kg of L-NMMA. This is consistent with previous reports that suggest higher potency of L-NAME compared with L-NMMA.^{29,33} Many times, to balance this higher potency, studies use a lower dose of L-NAME (eg, 2 mg/kg). Our data suggests that the response at this lower dose of L-NAME are similar to that with L-NMMA (4.25 mg/kg).

In Figure 5, R_2^* values typically show a temporal variation during NOSi infusion and in most cases the values fell back toward baseline before the end of the infusion. This observation was consistent with that of in rats. All individuals exhibited a positive BOLD response in the medulla, and a smaller response in the cortex. Note that the baseline values for R_2^* and MAP obtained in each individual on 2 different days were comparable and showed no statistically significant difference. Also, note in Figure 6, 1 subject showed anomalously high baseline R_2^* values on both occasions compared with others in both the medulla and cortex, although the response (R_2^*) was comparable to others. The subject was similar in characteristics (age, race, and gender) compared with other members in the group and was retrospectively confirmed to have not taken any medications on the days of the study.

We observed that L-NAME produced a dose-dependent change in R_2^* and MAP. The magnitude of peak change in R_2^* in humans induced by 2 doses of L-NAME is comparable to that in rats. The peak change in MAP in humans with 2 mg/kg is comparable to that in rats; however, the change in MAP in humans with 4 mg/kg is lower compared with rats. It should be noted that measurement conditions are slightly different between the rats and humans. The rats were anesthetized. Although blood pressure measurements in rats were obtained with a invasive arterial line, the measurements in humans were obtained with noninvasive approach. Although the equipment is MRI compatible, there is no data available on accuracy and precision of MAP measurements obtained during MRI.

A limitation of the present study is the lack of a time-control series [ie, additional group of control animals where no intervention (NOSi) was used]. Ideally BOLD MRI and invasive probe measurements should be performed quasi-simultaneously.

In conclusion, in rats our results show a dose-dependent change of R_2^* in response to L-NAME infusion. Compared with previous findings using bolus administration, the peak change in R_2^* measured with the 10 mg/kg dose was smaller when administered as an infusion.^{23,24} The data also suggest that changes in R_2^* are in part because of changes in blood flow. These data support the feasibility of observing changes after L-NAME administration in humans even at lower doses and when administered as slower infusion rate.

In humans, our preliminary results do support the feasibility of evaluating renal responses by BOLD MRI to NOS inhibition although their interpretation may be more difficult compared with rats (because of potential dependence on age, medication, hydration status, etc.). This in turn should allow for studies comparing responses to NOSi in subjects with hypertension (similar to our previous observations in rat kidneys²⁴) and ultimately in subjects at risk for developing hypertension, eg, familial history, race (African American vs. whites), patients with diabetes, etc.

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FIGURE 1.

Effect of L-NAME on the BOLD MR images. Images from 1 representative rat from each L-NAME dose group: 2 mg/kg, 4 mg/kg, and 10 mg/kg. Shown are anatomic, pre- and post-L-NAME R₂* maps obtained in the same slice position and displayed with the same window and level settings. Note the renal medulla in the pre R₂* map is relatively brighter than the cortex, indicating that the renal medulla has a lower baseline oxygenation level. Further increased brightness in the medulla in the post L-NAME R₂* map signifies a further reduction in oxygenation level. Changes in BOLD signal R₂* show a dose dependent response.

FIGURE 2.

Summary of blood pressure (A), medullary R_2^* (B), renal medullary pO_2 (C), and blood flow (D) data obtained in 6 rats at each dose of L -NAME. L -NAME infusion started at time 0. Note that MAP, pO_2 , and BF data were from the same groups of animals. BOLD data were from another group of animals. All measured parameters show a dose response. L -NAME infusion resulted in increased MAP and decreased renal medullary pO_2 and blood flow. Although medullary R_2^* increased consistently with the reduced medullary pO_2 and blood flow, a trend toward baseline was observed even during the infusion of L -NAME. All time points are statistically significant compared with the baseline based on Student t -test, except the few time points marked with "N." Error bars represent standard errors.

FIGURE 3.

Summary of individual BOLD peak responses (change in R_2^*) in renal medulla to NOS inhibitors in 18 SD rats. Each dose group included 6 rats. The medullary R_2^* increased after the NOS inhibition in each individual animal. The averaged R_2^* peak change during NOS inhibition compared with baseline is 10.7% with 2 mg/kg dose, 16.6% with 4 mg/kg dose, and 29.9% with 10 mg/kg dose. The response in the 3 dose group all show statistical significance based on Student *t*-test.

FIGURE 4.

A representative data set: anatomic image of the kidney (top), together with R_2^* maps from the same slice, showing the effect of NOS inhibition. At the center is the baseline R_2^* map, and at the bottom the R_2^* map acquired 18 minutes after the L-NMMA infusion (peak response). Both maps are displayed with the same window and level settings. Note that the medulla is relatively brighter than the cortex on the baseline R_2^* map, indicating lower basal oxygenation. Its brightness increases with L-NMMA administration, implying a further reduction in oxygenation in response to NOS-inhibition.

FIGURE 5.

Time course of R_2^* response to L -NMMA (obtained in the same subject as in Fig. 1). L -NMMA was administered at time zero. Note the small but significant change in the medullary R_2^* value over the measurement period with the increase of MAP, whereas the cortex shows no appreciable change. The error bars indicate the standard deviation in each ROI measurement. Medullary R_2^* increases gradually during L -NMMA infusion and achieves the maximum R_2^* at during L -NMMA administration.

FIGURE 6.

Summary of individual BOLD responses (change in R_2^*) in the renal medulla (A) and cortex (B) to NOS inhibitors in 13 subjects. Seven of them participated in the L-NMMA study. Six of them participated in the 2 L-NAME studies on different days with different L-NAME doses. The medullary R_2^* increased after the NOS inhibition in each individual, whereas cortex showed minimal change. The baseline R_2^* values with 2 L-NAME doses on 2 different days are very similar. Note a higher baseline R_2^* value in 1 subject on both the L-

NAME studies. The 4 mg/kg L-NAME infusion produced a larger BOLD response in the medulla compared with the 2 mg/kg L-NAME and 4.25 mg/kg L-NMMA.

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FIGURE 7.

Summary of individual peak MAP responses to NOS inhibitors in 13 subjects. Seven of them participated in 1 L-NMMA study. Six of them participated in 2 L-NAME studies on different days with different doses. The average baseline MAP value is similar in L-NAME group where the measurements were performed on 2 different days.

TABLE 1

Summary of Blood Flow, pO₂, and MAP Measurements in Rats (Mean ± SE)

Infusion Rate	Baseline			Peak Response		
	MAP (mm Hg)	pO ₂ (mm Hg)	Blood Flow (BPU)	MAP (mm Hg)	pO ₂ (mm Hg)	Blood Flow (BPU)
2 mg/kg/30 min	111.9 ± 12.3	38.1 ± 2.2	637.4 ± 102.8	127.4 ± 10.9	26.7 ± 3.1	501.0 ± 83.8
4 mg/kg/30 min	99.7 ± 6.8	36.6 ± 2.5	669.3 ± 76.8	132.0 ± 7.3	20.7 ± 3.6	428.9 ± 51.3
10 mg/kg/30 min	103.9 ± 3.1	33.9 ± 2.3	613.6 ± 111.2	148.4 ± 5.7	13.6 ± 2.7	386.4 ± 56.2

Six rats in each dose group.

TABLE 2Peak Changes (% of baseline) During L-NAME Infusion (Mean $\pm$ SE)

Infusion Rate	Medullary R2* (s ⁻¹)	Cortical R2* (s ⁻¹)	MAP (mm Hg)
4.25 mg/kg/ L-NMMA	113.6 $\pm$ 1.8	105.2 $\pm$ 1.8	112.0 $\pm$ 0.9
2 mg/kg L-NAME	110.9 $\pm$ 2.2	110.8 $\pm$ 2.4	114.2 $\pm$ 3.2
4 mg/kg L-NAME	119.2 $\pm$ 3.0	115.5 $\pm$ 3.7	117.9 $\pm$ 2.6

2 mg/kg L-NAME has similar effect on medullary R2* and MAP as that of the 4.25 mg/kg L-NMMA. Change is from baseline to peak.