Original Article

The Effect of Intra-Renal Administration of L-NAME on Renal Medullary and Cortical Blood Flow of Stroke-prone Spontaneously Hypertensive Rats and Wistar Rats

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Abstract

Background: Autocrine and paracrine factors produced within the kidney regulate the cortical and medullary blood perfusion (CBP and MBP, respectively), which include endothelins, prostaglandins, reactive oxygen species, and nitric oxide (NO). This study investigated the role of NO in regulating the CBP and MBP of a kidney in both stroke-prone spontaneously hypertensive rats (SHRSP) and Wistar rats. **Materials and Methods:** Groups (n = 10 for each) of male SHRSP and Wistar rats (250–300 g) were prepared for experiments. Two laser Doppler microprobes were inserted, 1.5 and 4.0 mm, into their kidneys to measure CBP and MBP before and after the intrarenal infusion of L-NAME, the NO synthase inhibitor at dose of 10 μg/kg/min. At the end of the experiments, the animals were killed with an anesthetic overdose. Data ± standard error of the mean were subjected to Student's t-test and significance taken at $P < 0.05$. **Results:** Interstitial infusion of L-NAME into the corticomedullary border (CMB) causes significant reduction in MBP in both SHRSP and Wistar rats by 18% ±5% and 12% ±4%, respectively. The magnitude of reduction is closely similar in both strains. Acute infusion of L-NAME into CMB has no effect on CBP but increases the blood pressure (BP) in both strains equally ($P < 0.05$). **Conclusion:** These results suggest that NO plays an important role in regulating the tone of medullary blood vessels in both hypertensive and normotensive states with similar extent. L-NAME can easily spread into the systemic circulation, which is evidenced by the increase of BP.

Keywords: L-NAME, medullary blood perfusion, renal hemodynamics, spontaneously hypertensive rats

Introduction

The kidney function is important in controlling the normal level of blood pressure and considered to be one of the most important causes for the development of essential hypertension once its principal function is disturbed.[1,2]

In spontaneously hypertensive rats (SHR), the renal vascular tone is elevated when compared with normotensive rats.[3-5] The reason behind enhancement of renal vascular tone in hypertensive animals has not yet been fully discovered. A few studies on genetically hypertensive rats suggest that an increase in response to a vasoconstrictor such as angiotensin II might be the reason. Others suggest that the elevated level of oxidative stress and particularly the level of reactive oxygen species play a major role in the pathogenesis of hypertension in humans as well as in different models of hypertension, the SHR is one among them.

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Renal medullary blood flow might affect the regulation of BP majorly and greatly mediate the pressure natriuresis.[6,7] Furthermore, it has been shown that reduction in MBP leads to the development of hypertension,[8] while an increase in the flow prevents against the increase in BP.[9]

The endogenously formed nitric oxide (NO) participates in control of renal MBP, water and sodium excretion, and arterial BP. Chronic inhibition of endogenous NO in the renal medulla has been reported to markedly decrease MBP and sodium excretion and consequently result in a rise of arterial BP in rats.[8,9]

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Oxidative stress implies that ROS are produced in high numbers in relation to metabolism.[10] Defense against the excessive rise in ROS is provided by superoxide dismutase enzyme (SOD) that scavenges superoxide anion, which then may allow improvement in the action of NO by increasing the availability of NO that will lead to a vasodilation.

Cowley *et al*., 1995 suggested that in hypertensive rats, renal MBP was lower in comparison to normotensive rats, but the cortical flows were the same.[11] The authors also suggested that MBP in hypertensive rats reduced in comparison to that of the normotensive rats even before the appearance of hypertension. This apparent reduction in MBP was accompanied by increased vascular tone in the afferent arterioles of juxtaglomerular nephrons, with prolonged hypertension the cortex can also be involved in the development of glomerular lesions and renal vascular degeneration.[11]

The superoxide anions may react to NO and cause inactivation for NO[12,13] and may lead to endothelial dysfunction,[14] this by generation of highly oxidative and nitrosating species, including peroxynitrite (ONOO), which is known as a vasoconstrictor.[15,16] The superoxide reacts with NO three times faster than its reaction with SOD, which determines the biological action of NO; so, in the hypertensive rats where the level of superoxide is elevated, we may observe a different activity and role for NO due to the reduction in the half-life of NO by superoxide anions in this strain.[17,18]

In this study, we tested whether the acute blockage of local intrarenal NO synthase inhibitor (NOS) enzyme affects basal medullary and cortical blood flow levels and the findings were compared between stroke-prone SHRs (SHRSP) and normotensive Wistar rats.

Materials and Methods

All experimental procedures were performed under the European Community Directive 2010/63/EU and were approved by the local animal experimentation ethical committee at University College Cork.

Four groups of male SHRSP and Wistar rats, 250–350 g (~12 weeks old), were obtained from Harlan (Bicester, UK) and maintained under a 12 h–12 h light–dark regimen at 20°C ± 3°C in the Biological Services Unit, University College Cork.

Surgical protocol

Rats were made to fast overnight but were given access to water. Anesthesia was induced with 1 ml of a chloralose–urethane mixture (16.5 and 250 mg/ml respectively, I. P.) and maintained using bolus I. V. doses of 0.05 ml every 30 min. A tracheostomy was carried out to ensure a patent airway. Cannulae were inserted into the right femoral vein to facilitate the infusion of sustaining saline (3 ml h of NaCl 9 g/l) and the right femoral artery to permit the measurement of mean arterial pressure (MAP) and heart rate (HR).

The kidney was exposed by a flank incision and prepared as described before.[19,20] A small cannula was inserted approximately 4.5 mm into the rostral pole of the kidney to allow saline or L-NAME (a dose of 10 μg/kg/min) to be infused at 1 ml/h into both cortex and medulla.[19,21]

Two optical fiber microprobes (MT B500-0 L120, 0.5 mm diameter, Perimed CE 0413, Sweden) were inserted gently into the kidney to depths of 1.5 mm and 5.0 mm to measure cortical and medullary blood perfusion (CBP and MBP), respectively. The flow probes were connected to a laser-Doppler flow meter (Perifle × 4001 Master, Perimed, Sweden).

A 1.5-h postsurgical stabilization period was given before the commencement of all experiments. On completion of each experiment, animals were euthanized by anesthetic overdose, and the kidneys were sectioned to confirm the location of the flow probes.

Experimental groups and protocol

Control groups

The control groups ($n = 10$) received the vehicle, which was the solution in which the drugs were dissolved. The vehicle was normal saline, 0.9% NaCl, which was made by dissolving 9 g of sodium chloride (Sigma-Aldrich Company, Switzerland) in 1 liter distilled water. The vehicle was infused into corticomedullary border (CMB) at a rate of 1 ml/h in this group.

L-NAME groups

This group ($n = 10$) received L-NAME, N-nitro-L-arginine methyl ester (Sigma-Aldrich Company, Germany), a NO synthase inhibitor; the compound was dissolved in normal saline and then infused into CMB at the rate of 1 ml/h. This dose was selected based on a previous report from our laboratory where the same dose had been used and found to cause a reduction in medullary blood perfusion.[19,21]

After a 90-min period of stabilization, a baseline recording for CBP, MBP, MAP, and HR were obtained over the 5-min period before the start of the renal interstitial infusion. Vehicle (saline) or L-NAME was infused for 60 min, after which a further set of readings were taken for over 5 min while the infusion continued.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). The SEM was used as a measure of data dispersion. The significance of changes was evaluated by pairing Student's *t*-tests within the groups. For intergroup comparisons, classical two-way analysis of variance, followed by Tukey's test, was used. Significance was accepted when $P < 0.05$.

Results

Effect of intrarenal infusion of vehicle on cortical blood perfusion and medullary blood perfusion in stroke-prone spontaneously hypertensive rats versus Wistar rats

Initial experiments were required to determine the effect of a 1 ml/h infusion of saline into the CMB on baseline renal regional blood flow.

The rats were in a stable condition with constant BP and HR, which were maintained throughout the whole experiment and the baseline readings for MAP, HR, CBP, and MBP are summarized in Table 1.

There is no significant alteration in the cortical or medullary blood perfusion after intramedullary infusion of vehicle over a period of time compared with the baseline values in both normotensive and hypertensive groups (the data not shown).

It was noted that the basal level of MAP in the SHRSP was significantly higher than that observed in Wistar rats ($P < 0.05$). The values in both strains before the infusion of saline were (MAP in SHRSP = 136 ± 4 mmHg, MAP in Wistar = 108 ± 4 mmHg; $n = 10$; $P < 0.05$) and after the saline infusion (MAP in SHRSP = 131 ± 5 mmHg, MAP in Wistar = 104 ± 6 mmHg).

In the SHRSP, renal medullary interstitial infusion of vehicle into CMB had no significant effect on either CBP (154 ± 5 PU vs. 159 ± 5 PU; $n = 10$) or MBP (56 ± 4 PU vs. 55 ± 5 PU; $n = 10$; data not shown).

The CBP of SHRSP was close to the average CB*P* values in Wistar rats and was also relatively stable over the period of intramedullary infusion of vehicle. However, it was notable that MBP was significantly lower in SHRSP in comparison to Wistar rats (56 ± 4 PU vs. 81 ± 8 PU, $n = 10$; $P < 0.05$); CBP and MBP in Wistar rats were similarly unaffected by intramedullary infusion of vehicle.

Effect of renal medullary interstitial infusion of L-NAME on cortical blood perfusion and medullary blood perfusion in stroke-prone spontaneously hypertensive rats versus Wistar rats

The baseline data for MAP, HR, CBP, and MBP in these groups of experiments are summarized in Table 1. It can be seen that L-NAME (10 μg/kg/min) significantly increased the MAP in both SHRSP and Wistar rats [SHRSP = 130 ± 3 mmHg before L-NAME, 144 ± 4 mmHg after L-NAME; $P < 0.05$, $n = 10$: Wistar = 110 ± 4 mmHg before L-NAME, 124 ± 3 mmHg after L-NAME; $P < 0.05$, $n = 10$, Figure 1]. Although HR remained stable in both groups.

Renal interstitial infusion of L-NAME into CMB caused no significant changes to CBP in both SHRSP and Wistar rats.

In contrast to the effects on CBP, infusion of L-NAME into CMB decreased MBP in the SHRSP rats by 18% ±5% ($P < 0.05$, $n = 10$). This L-NAME-induced reduction in MBP was greater than that seen in Wistar rats (12%±4%, n = 8, $P < 0.05$), but there was no significant difference in the magnitude of the reduction between the two strains [Figure 2].

Discussion

The SHRSP is now widely accepted as a good animal model of genetic hypertension.[22] In this respect, it displays characteristic symptoms of diseases such as increased peripheral resistance, atherosclerosis, nephrosclerosis, and associated renal structural changes, which are comparable to the hypertension-associated pathology in humans.[23,24] Furthermore, the hypertensive characteristics displayed by SHRSP also exacerbate with age, which also mirrors the human condition.[23,25]

As hypertension has been associated with renal damage, the study of genetic models of hypertension and their corresponding renal function and reaction to renal ischemia is of the upmost importance.[26] Therefore, the SHRSP, the model of hypertension, was selected to investigate the impact of hypertension on renal perfusion and function before and after a period of intramedullary infusion of drug that inhibited enzyme involved in reducing the level of oxidative stress in the kidney.

Control groups

These groups were used to evaluate the BP, HR, MBP, and CBP responses to the infusion of vehicle into the CMB in both SHRSP and Wistar rats, and these groups were considered as control groups against the other groups that received a drug mixed with the vehicle, so we could judge whether the effect originated from the drug or from the vehicle. It was evident

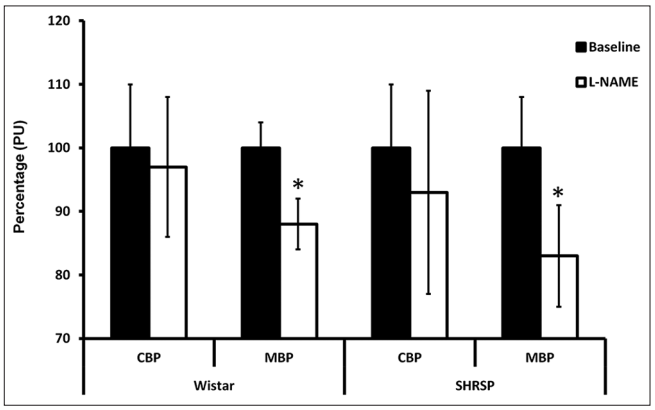

Figure 1: Histogram demonstrating the effect of L-NAME on CBP and MBP in Wistar rats relative to SHRSP ($n = 10$ for each data point). Data analysis was performed using Student's paired *t*-test within the group and two-way ANOVA between the groups. *Significant difference between the L-NAME treated group and the respective baseline ($P < 0.05$) (at column width)

Table 1: Baseline values of mean arterial pressure, cortical blood perfusion, medullary blood perfusion, and heart rate

Parameter	Wistar		SHRSP	
	Vehicle	L-NAME	Vehicle	L-NAME
n	10	10	10	10
Baseline MAP (mmHg)	108±4	114±3	136±4*	130±3*
Baseline CBP (PU)	150±19	112±11	154±5	115±12
Baseline MBP (PU)	81±8	76±3	56±4*	65±5*
Baseline HR (beats/min)	374±17	321±17	268±23*	237±16*

Values obtained from vehicle and L-NAME treated groups of SHRSP and Wistar rats before any infusion. Statistical analysis was performed using a two-way ANOVA test. *$P<0.05$, when baseline values of SHRSP were compared with those of Wistar rats. MAP: Mean arterial pressure, CBP: Cortical blood perfusion, MBP: Medullary blood perfusion, HR: Heart rate, SHRSP: Stroke-prone spontaneously hypertensive rats

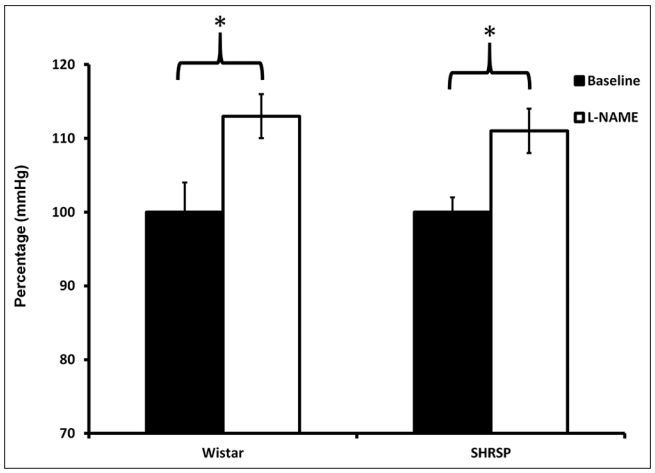

Figure 2: Histogram demonstrated the effect of L-NAME on BP of SHRSP and Wistar rats. * indicates $P < 0.05$, significantly different from the baseline ($n = 10$ for each data point). Statistical analysis was performed using Student's paired *t*-test within the group and two-way ANOVA between the groups (at column width)

that over the periods of measurements, perfusions and the other parameters remained relatively unchanged in each group indicating that the preparation was stable, and the vehicle had no action on all the parameters.

There were significant differences between the baselines of cortical perfusion and medullary perfusion. The cortex was shown to have a higher level of red cell flux than that obtained from the medulla, which is in accordance with the understanding that the renal cortex has a greater concentration of blood vessels and therefore a higher perfusion than the medulla.

The previously mentioned findings were consistent with observations from earlier studies on Wistar and SHRSP in our laboratory[19,21,27] that findings plus the stability of all the parameters over the whole period of the control study provided a strong evidence regarding the reliability of our current experiments.

The second part of this study was to examine the effect of the acute inhibition of the NOS enzyme and analyze the outcome to assess its underlying contribution to tone of the renal vasculature in SHRSP and Wistar rats.

L-NAME groups

This study set out to investigate the role of NO in the kidney, particularly on the renal cortical and medullary microvasculature, as NO has been characterized as having potent vasodilator actions and also has been reported to be generated in significant amounts in the renal medulla. It is known that the kidney possesses three distinct NO synthase (NOS) isoforms (iNOS, eNOS, and nNOS); but in this study, a nonselective NO inhibitor was chosen to inhibit the synthesis of NO from the amino acid L-arginine.

Previous studies examined the effect of systemic NO inhibition on systemic and renal hemodynamics and excretory function have yielded disparate results. There have been reports of increased, as well as unchanged, MAP after L-NAME administration.[28-30] The findings are more divergent in terms of renal hemodynamic and excretory function, ranging from an increase, decrease to no change.[28-31] The variability in these finding probably reflects differences in the experimental preparation, routes, and methods of L-NAME administration. In the present study, the possibility that NO generation may not be efficiently prevented was excluded since the dose of L-NAME used has been shown to be effectively block NOS activity in Wistar rat kidney and to cause an increase in BP and depressed renal plasma flow, after intravenous administration at least in same dose used in our study or higher.[19,28]

The effect of chronic intravenous infusion of L-NAME on BP and intrarenal blood flow had been previously studied and the authors provided clear evidence for the reduction in renal MBP and increase in systemic BP but there was no change in cortical perfusion;[8] the same findings were obtained previously from our laboratory when the L-NAME was administered locally into the CMB of Wistar rats;[19] also, these findings are in agreement with what we currently find in the new set of experiments on Wistar rats. However, the number of animals and the protocol were slightly different but still provided same findings, which indicate that NO plays an important role in regulating the tone of blood vessels in the medullary region.

In SRRSP, where the level of oxidative stress is considered to be very high due to the increased amount of superoxide anion in the medullary region and the increase in sensitivity of the medullary region of SHRSP to ROS,[27] the NO appears to play a similar role in maintaining the stability of blood perfusion in the medullary region of the kidney as same as their action on normotensive rats although a tendency of having more role in hypertensive state was obvious by reducing the MBP in SHRSP by 18% and by 12% in Wistar rats.

Numerous studies have demonstrated that endothelium-dependent vasorelaxation markedly decreases in the hypertensive state and in experimental animal models of hypertension.[32,33] Furthermore, exaggerated production of superoxide anion by the vascular wall has been observed in different animal models of hypertension including SHRSP.[34,35] There is a growing body of evidence that supports the possibility of increased oxidative inactivation of NO by an excess of superoxide and may account for the decrease in available NO and endothelial dysfunction observed in hypertensive rats.[33,36,37]

In the present study, we found that antagonizing NOS with L-NAME reduced MBP significantly in both SHRSP and Wistar rats. These findings suggested that the lower amount of NO found in the SHRSP plays a very important role in the control of vascular tone, which was to counteract the action of superoxide anions. It seems that a small part of the L-NAME given locally did get absorbed into the systemic circulation as it significantly increased the BP in both SHRSP and Wistar rats.

An interesting finding in the present study was the arterial pressure which increased on the acute infusion of L-NAME. The elevation of arterial BP in this case is probably due to a number of different factors; the L-NAME infusion may have initially increased BP because of increased peripheral resistance, as previously described by Gardiner *et al*.,[38] because we hypothesized that some of the L-NAME reached the systemic circulation. Other predictable reasons for the elevation of BP might be due to sodium and water retention, which has been reported previously,[8,39] while L-NAME given locally to the renal medulla would have effectively blocked the all NOS isoforms, which would prevent any local production of NO, which in turn would lead to vasoconstriction of the medullary blood vessels. This would also reduce the glomerular filtration rate and then reduce the sodium excretion from the kidney.

Conclusion

The acute intramedullary infusion of L-NAME into CMB prevented NO production that would cause a reduction in MBP, which may be important in the initiation of sodium and water retention, and leads to hypertension. Whereas cortical blood flow was unaltered by intramedullary L-NAME infusion. This study suggested that the availability of NO in the kidney region plays a major role in the control of renal microvasculature in both SHRSP and Wistar rats equally, regardless to the amount of ROS produced in the region.

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Conflicts of interest

There are no conflicts of interest.

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