Original Article

# The Role of Vasodilator Receptors of Renin–angiotensin System on Nitric Oxide Formation and Kidney Circulation after Angiotensin II Infusion in Renal Ischemia/Reperfusion Rats

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## Abstract

**Background:** Nitric oxide (NO) as a vasodilator factor has renoprotective effect against renal ischemia. The balance between angiotensin II (Ang II) and NO can affect kidney homeostasis. The aim of this study was to determine NO alteration in response to renin–Ang system vasodilator receptors antagonists (PD123319; Ang II type 2 receptor antagonist and A779; Mas receptor antagonist) in renal ischemia/reperfusion injury (IRI) in rats. **Materials and Methods:** Sixty-three Wistar male and female rats were used. Animals from each gender were divided into four groups received saline, Ang II, PD123319 + Ang II, and A779 + Ang II after renal IRI. Renal IRI induced with an adjustable hook. Blood pressure and renal blood flow (RBF) measured continuously. The nitrite levels were measured in serum, kidney, and urine samples. **Results:** In female rats, the serum and kidney nitrite levels increased significantly by Ang II ($P < 0.05$) and decreased significantly ($P < 0.05$) when PD123319 was accompanied with Ang II. Such observation was not seen in male. Ang II decreased RBF significantly in all groups ($P < 0.05$), while PD + Ang II group showed significant decrease in RBF in comparison with the other groups in female rats ($P < 0.05$). **Conclusion:** Males show more sensibility to Ang II infusion; in fact, it is suggested that there is gender dimorphism in the Ang II and NO production associated with vasodilator receptors.

**Keywords:** *Nitric oxide, ischemia/reperfusion injury, renin–angiotensin system, Ang II type 2, Mas receptor*

## Introduction

Nitric oxide (NO) is a vasodilator factor produced by NO synthase (NOS). It is known as renoprotective factor due to its anti-inflammatory, vasodilatory, and antioxidant properties against of renal ischemia/reperfusion injury (IRI).[1,2] Renal IRI is largely considered a reversible phenomenon,[3] and it enhanced and activated the expression of NOS proteins.[4] Studies have shown that NO and nitrite have cytoprotection properties in IRI models[5,6] and endothelium-derived NO has a reciprocate interaction with angiotensin II (Ang II).[7,8]

NO formation are affected through Ang II type 2 ($AT_2R$) and Ang 1–7 (Mas receptor [MasR]) receptors which they antagonize the effects of Ang II type 1 ($AT_1R$) receptor.[9,10] $AT_1R$ antagonists can increase the renal NO, and this increase can be attenuated by $AT_2R$ blockade.[11,12] Ang 1–7 through MasR causes vasodilation through excitation of endothelium-dependent NO release.[10] It can stimulate the production of the NO and prostaglandin,[13] thereby be a physiological antagonist of Ang II.[14] It is important that integrity of kidney system can be maintained with balance between NO and Ang II.[15]

Furthermore, it is known that sex differences exist in kidney IRI.[16] It has been made clear that males are more susceptible to renal IRI than females,[16,17] and renal function disturbance is gender related.[18,19] Accordingly, we hypothesized that renal blood flow (RBF) response to Ang II is associated with NO production from one side and it is related to interaction between receptors activity by the other side. Therefore, the aim of the study was to determine RBF and NO response to Ang II when vasodilator receptors were blocked in male and female rats subjected to IRI.

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## Materials and Methods

### Animals

A total of 63 male and female Wistar rats (respectively weighed 211.8 ± 0.9 g, $n$ = 29 and 185.6 ± 0.6 g, $n$ = 34) were used in this experimental study.

### Renal ischemia reperfusion and catheterization

Rats were anesthetized with 1.7 g/kg urethan (Sigma, St. Louis, MO, USA), trachea was isolated to insert air ventilation tube, and also catheters were implanted into the left carotid and femoral arteries and jugular vein. An adjustable hook (as clamp) was placed around the abdominal aorta (above renal arteries) to induce renal IRI and also adjust renal perfusion pressure (RPP) in base levels during infusion of Ang II. Blood pressure was monitored through carotid artery, and following surgical process, RPP was set at 25 mmHg through tightening the abdominal aortic clamp to induce renal IRI for 30 min and then allowed to reperfusion by loosening it. The left kidney was exposed and fixed in adjustable cup. Renal artery was separated from the renal vein. The ultrasound flow probe interfaced with a compatible flowmeter (T108; Transonic Systems) was hooked around the renal artery to measure RBF directly.

### Group design

Each male or female group was divided into four subgroups (total eight subgroups). In summary, the designed groups were as following:

1. Group 1: Male or female rats treated with vehicle; saline (as solvent for antagonist and Ang II)
2. Group 2: Male or female rats treated with vehicle for antagonist, and then Ang II was infused
3. Group 3: Male or female rats treated with $AT_2R$ antagonist; PD123319 (Sigma, St. Louis, MO, USA), and then Ang II was infused
4. Group 4: Male or female rats treated with MasR antagonist; A779 (Bachem, King of Prussia, MO, USA) and then Ang II was infused.

### Experimental protocol

At the beginning of reperfusion, the antagonist was administered as bolus dose of 50 μg/kg followed by continuous infusions at 50 μg/kg/h for A779 and bolus doses of 1 mg/kg followed by continuous infusions at 1 mg/kg/h for PD123319 using microsyringe pumps (New Era Pump Systems Inc., Farmingdale, NY, USA) and jugular vein tube. The antagonists' infusions were continued during the experiment to the end. After 30 min commencing vehicle or antagonist treatments, intravenous Ang II infusion (500 ng/kg/h) started for 45 min. At the end of experiment, the blood and urine samples were obtained after the vehicle or Ang II infusion for nitrite concentration determination using a colorimetric assay kit (Promega Corporation, USA) that involves the Griess reaction.

### Statistical analysis

Data are expressed as mean and standard error mean. SPSS software version 20 was used to analyze the data. The serum, tissue, and urine levels of nitrite, RBF, RPP were compared through ANOVA between the groups, and LSD was used as a posttest to find the significant difference between each two groups. $P \leq 0.05$ was considered to be statistically significant.

## Results

### Effect of angiotensin II on serum, kidney, and urine nitrite levels in the presence of A779 or PD123319 or both

In female rats, the serum and kidney tissue levels of nitrite increased significantly by Ang II infusion alone ($P < 0.05$), but Ang II-induced NO production decreased when PD123319 or A779 was accompanied with [Figure 1] so that there is significant difference between Group 2 and 3. Such observation was not seen in male [Figure 1].

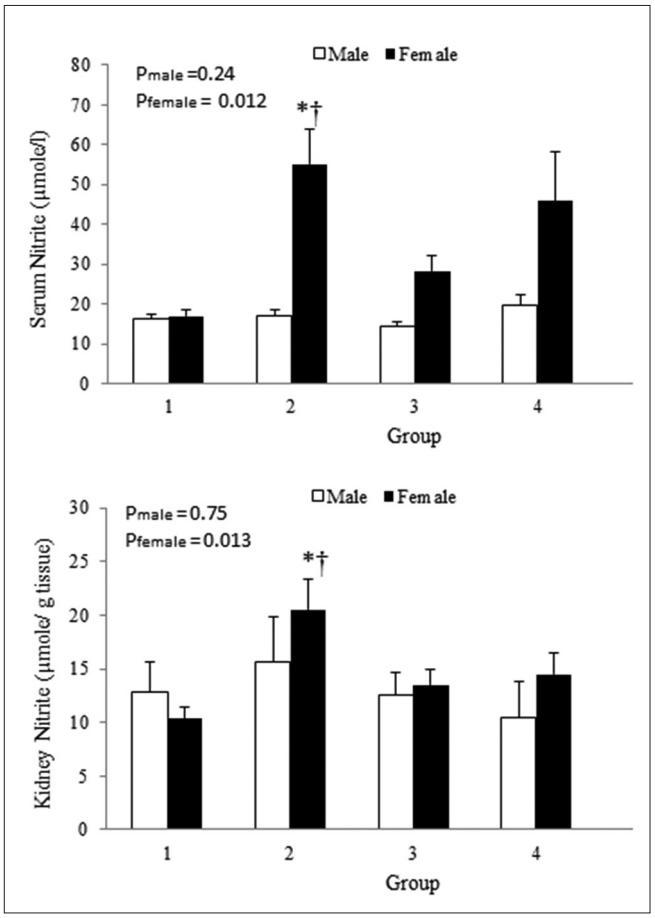


**Figure 1: Serum and kidney nitrite levels in male and female rats. *Significant difference from control group ($P < 0.05$); †Significant differences from Group 3 in same gender ($P < 0.05$). Group 1 (control): Rats treated with vehicle; saline. Group 2: Rats treated with vehicle for antagonist, and then angiotensin II was infused. Group 3: Rats treated with angiotensin II type 2 antagonist; PD123319 and then angiotensin II was infused. Group 4: Rats treated with Mas receptor antagonist; A779 and then angiotensin II was infused**

There is no difference in urine nitrite concentration between male or female groups [Table 1].

### Effect of angiotensin II on renal blood flow in the presence of A779 or PD123319 or both

Ang II infusion decreased RBF in female and male rats significantly at constant RPP ($P < 0.05$) [Figure 2]. In addition, PD + Ang II group showed significant decrease in RBF was observed when PD123319 was accompanied with Ang II [Figure 2].

**Table 1: Urine nitrite level (μmol/L)**

| Groups | Female | Male | *P* |
|---|---|---|---|
| 1 | 9.47±1.36 | 8.37±1.58 | 0.43 |
| 2 | 11.32±1.47 | 9.13±1.40 | 0.31 |
| 3 | 9.36±0.62 | 9.14±0.73 | 0.90 |
| 4 | 11.25±1.94 | 10.40±1.12 | 0.83 |
| ANOVA (*P*) | 0.51 | 0.27 | - |

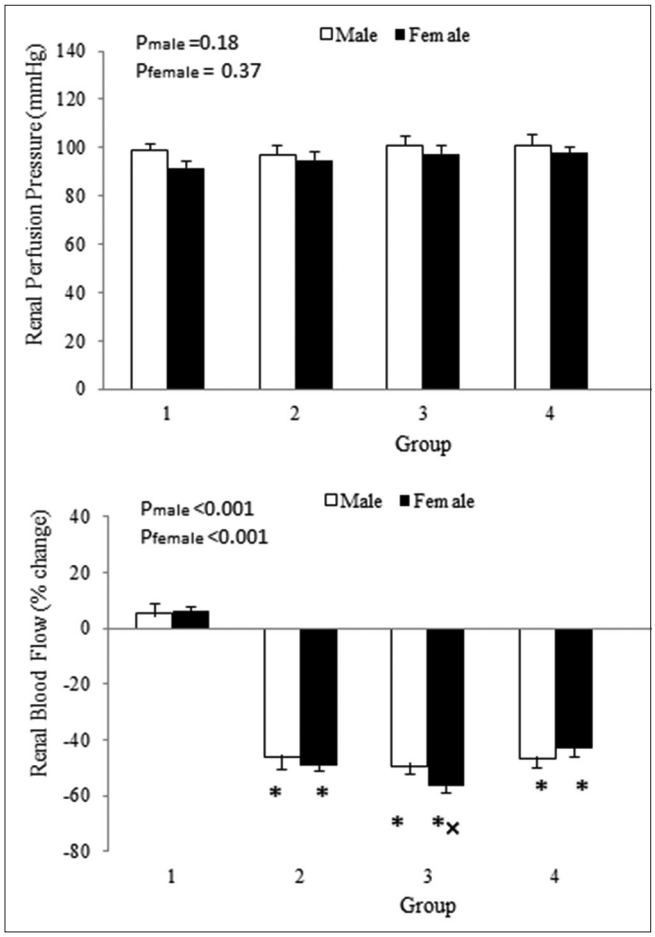


**Figure 2: Renal perfusion pressure and the percentage change of renal blood flow in male and female rats. * Significant difference from Group 1 ($P < 0.05$) in similar gender; ×Significant differences from Groups 2 and 4 ($P < 0.05$). Group 1: Rats treated with vehicle; saline. Group 2: Rats treated with vehicle for antagonist, and then angiotensin II was infused. Group 3: Rats treated with Ang II type 2 antagonist; PD123319 and then angiotensin II was infused. Group 4: Rats treated with Mas receptor antagonist; A779 and then angiotensin II was infused**

## Discussion

Increased glomerular NO synthesis in renal IRI seems to be a protective mechanism that counteracts vasoconstrictor and inflammatory phenomena occurring during the reperfusion period. These phenomena play a major role to impair the recovery of renal function after ischemia or after renal transplant.[20] In renal IRI, nitrite was not changed significantly after Ang II infusion in male rats; however, in age-matched female rats, it was increased significantly. However, when $AT_2R$ or MasR was blocked, nitrite level was reduced in serum. As we know, males show more sensibility to Ang II infusion; in fact, it is suggested that there is gender dimorphism in the Ang II activity and reported that males are more responsive to Ang II in isolated aorta rings and mesenteric microvessels than females.[21] Furthermore, it was reported a gender dimorphism in NO system including premenopausal women make more total NO than men[22] and there is a greater abundance of the constitutive NOS in young adult kidney of female than male rat.[23,24] It was showed that estrogen exerts marked stimulatory actions on endothelial NO levels, and in female rat, the active life of NO is prolonged probably due to sex-related antioxidant actions.[25] In accordance with us, it is demonstrated that testosterone induces inhibition of NO-dependent vasodilation,[25] and also, there is an upper ratio of $AT_2R/AT_1R$ of Ang II in blood vessels and kidney in females compared with males.[21,26] Despite lesser findings about the endothelial Ang II signaling, some studies suggest that the endothelial Ang II signaling positively, as well as negatively, regulates NO signaling pathway and so that modulates endothelial dysfunction.[27] More evidence suggests the role of the $AT_1R$ in regulating the balance between NO and reactive oxygen specious through endothelial signaling.[28,29] Endothelial dysfunction, characterized by less production of NO as well as NO bioavailability, leads to accelerated vasoconstriction,[28,30] Reckelhoff reported that increased activity of Ang II stimulates superoxide production through nicotinamide adenine dinucleotide phosphate oxidase, and due to the stimulatory actions of testosterone, this effect is more prominent in the male than female.[23] Female hormones that stimulate NO production exert an inhibitory action on Ang II.[27] In addition, it is observed that there are increased medullary NOS and NOS in whole kidney homogenates in female than male.[24,31] $AT_2R$ is thought to be associated with the vasodilatory actions of Ang II, which may be mediated by NO.[11] A lower ratio of $AT_1R/AT_2R$ was reported in female compared with male in blood vessels and kidneys.[21] Furthermore, male showed a lower expression of $AT_2R$ in kidneys compared with female.[21] As we observed, the same situation for nitrite is existed with MasR blocker infusion in female versus male but nonsignificantly. In consistence with us, other research reported that the expression and activation of the MasR differ between the sexes, and they have revealed

greater renal $ACE_2$ and MasR gene expression in female as compared to male normotensive rats,[32-34] and therefore, in female, the balance tends to renin angiotensin sysytem (RAS) stimulation toward the depressor arm.[34] In the nonrenal vasculature, Ang 1–7 increased production of NO through Mas involved pathways.[35] Ang 1–7 induced NO release through stimulating endothelial NOS (eNOS) and these effects were blocked by the A779[35] so that is reasonable the NO production decreased compared to Ang II infusion group in female rats. However Ang II acts through different receptors ($AT_1R$ and $AT_2R$), so the effect of Ang II in RBF is dominant and Mas blockade could not change RBF reduction induced by Ang II. Other research also indicated the different response to Ang II related to MasR between male and female.[36] In this study, we did not observe any difference in nitrite level of urine in male or female groups. We did not normalized nitrite levels for the urine volume per time unit, and therefore, this limitation may be the cause.

## Conclusion

NO and RBF responses to Ang II administration depend on vasodilator receptors of RAS in a gender-related manner.

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### Conflicts of interest

There are no conflicts of interest.

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