

## Mas receptor antagonist (A799) alters the renal hemodynamics responses to angiotensin II administration after renal moderate ischemia/reperfusion in rats: gender related differences

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### Abstract

Moderate renal ischemia/reperfusion (I/R) injury is one of the major causes of kidney failure. We examined the role of Mas receptor (MasR) antagonist (A779) alone and combined with angiotensin II (Ang II) type 2 receptor (AT2R) antagonist (PD123319) on renal hemodynamic responses to Ang II after moderate I/R in male and female rats. Anaesthetized Wistar rats underwent 30 min partial ischemia by reduction of renal perfusion pressure (RPP) and subjected to block vasodepressor receptors followed by Ang II (100 and 300 ng/kg/min) infusion. Mean arterial pressure (MAP), renal blood flow (RBF), and renal vascular resistance (RVR) responses were assessed during graded Ang II infusion at controlled RPP. Thirty min post reperfusion, the Ang II infusion reduced RBF and increased RVR in a dose-related fashion ( $P < 0.05$ ). However, A779 alone or A779 plus PD123319 infusion increased the RBF and RVR responses to Ang II infusion significantly ( $P < 0.05$ ) in female but not in the male rats. MasR antagonist altered the RBF and RVR responses to Ang II infusion in female, and these responses were not altered statistically in dual blockade of MasR and AT2R. These findings suggest the important role of Mas receptor in renal vascular response to Ang II in female rats after moderate I/R.

**Keywords:** Angiotensin II; Ischemia/reperfusion; Mas receptor; Renal blood flow; Renal vascular resistance.

### INTRODUCTION

Moderate ischemia/reperfusion (I/R) injury is a common renal disturbance in clinic, and some evidences indicated that reduction in kidney perfusion could be associated with acute kidney injury (1,2). I/R injury may result from cardiac arrest, shock, renal artery stenosis, and some surgical interventions such as aortic cross-clamping, partial nephrectomy, and renal transplantation (1-3). I/R injury alters the vascular response (4,5), and it may also cause to produce reactive oxygen species (ROS), chemokines, cytokines, leukocytes malicious, and harmful activation which disturb renal blood flow (RBF) (6) and endothelial function (7).

In addition, the I/R injury may be gender related (8,9). It was reported that glomerular

injury (10), hypertension (9), polycystic kidney disease (11), and renal ischemia (8) include gender dependent outcomes. In fact females are more resistant to I/R-induced kidney injury (12). On the other hand renin-angiotensin system (RAS) also plays an important role in kidney circulation and renal I/R injury, and this system also acts gender dependently (13-15).

Angiotensin II (Ang II), the main biologically active component of RAS, interacts with Ang II type 1 receptor (AT1R) to induce renal vasoconstriction, and it also binds to Ang II type 2 receptor (AT2R) to reduce vascular resistance.

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Ang 1-7 is the other component of RAS that is primary derived from Ang II and the Mas receptor (MasR) has been identified as a functional receptor for Ang 1-7 (16). Some data related to the role of MasR in the renal vascular response to Ang 1-7 in male and female rats were reported (3,17), however, there is evidence that MasR could alter the function of AT2R in renal vascular response to AngII infusion (13).

We hypothesized that RBF and renal vascular resistance (RVR) responses to AngII administration after moderate renal I/R injury are gender related and involve MasR and AT2R. To test the hypothesis, either male or female rats underwent moderate renal I/R injury and the renal vascular responses to Ang II infusion were determined using MasR antagonist (A779) alone, and A779 plus AT2R antagonist (PD123319).

## MATERIALS AND METHODS

### *Animals*

Age matched male ( $211.4 \pm 1.0$  g,  $n = 22$ ) and female ( $185.6 \pm 0.8$  g,  $n = 24$ ) Wistar rats from the animal care center of Isfahan University of Medical Sciences, Isfahan, I.R. Iran, were housed in cages at the room temperature of  $25 \pm 1$  °C with 12 h light/dark cycle. This research was approved in advance by the Ethic Committee of Isfahan University of Medical Sciences (Ethical No. IR.MUI.REC.1393.3.735).

### *Surgical preparation*

The rats were anesthetized with urethane (1.7 g/kg i.p.; Merck, Germany). After tracheostomy, the left jugular vein was isolated, ligated distally, and catheterized with polyethylene tube for drugs infusion. Catheters also were implanted into the left carotid and femoral arteries attached to a pressure transducer and a bridge amplifier (Scientific Concepts, Vic., Melbourne, Australia) to measure mean arterial pressure (MAP) and renal perfusion pressure (RPP), respectively. The catheter in the femoral artery was inserted to reach the descending aorta, and pressure measurement at this point was considered as RPP (13-15). The animals were placed in a

lateral posture on a table equipped with heating lamp to maintain normal body temperature. To measure the RBF, left kidney was exposed and placed in a holder secured to the operating table and its artery was surrounded by a transit-time perivascular ultrasound flow probe (Type 2SB; Transonic Systems, Ithaca, NY, USA) interfaced with a compatible flow meter (T108; Transonic Systems). An adjustable clamp was placed around the abdominal aorta above renal arteries in order to induce renal moderate ischemia (renal hypo-perfusion) and also to adjust RPP in control levels during infusion of Ang II. Throughout the experiment the hemodynamic parameters were measured continually (13-15).

### *Experimental protocol*

#### *Antagonist infusion*

The animals were divided randomly into 3 groups of the males (groups 1-3) and 3 groups of the females (groups 4-6). Following surgical procedures, a 30-45 min period was allowed the animals to stabilize and baseline (control) data for MAP, RPP, and RBF were recorded. We considered the RPP around 25 mmHg as a moderate hypo-perfusion for the kidney, and to induce renal moderate I/R injury, RPP was set at  $23 \pm 2$  mmHg (moderate ischemia) via tightening the abdominal aortic clamp for a 30 min period then reperfusion was allowed by loosening the clamp. The antagonists or vehicle were infused as soon as reperfusion was begun, and the effect of antagonists or vehicle was determined at 30 min post agents infusion. The effects of vehicle (saline, groups 1 and 4), A779 (Bachem Bioscience Inc., King of Prussia, PA, USA) (groups 2 and 5) and A779 plus PD123319 (Sigma, St. Louis MI, USA) (groups 3 and 6) infusion were tested. In summary, after inducing renal moderate I/R the animals were treated as follows:

Group 1 ( $n = 8$ ), male rats treated with vehicle for antagonist, and the vascular responses to Ang II (Sigma, st Louis MI, USA) infusion were determined; group 2 ( $n = 6$ ), male rats treated with A779, and the vascular responses to Ang II infusion were determined; group 3 ( $n = 8$ ), male rats treated with A779 plus PD123319, and the vascular responses to Ang II infusion were determined;

group 4-6 (n = 8 in each group), female rats received the same regimen as male rats in the groups 1-3 respectively. The antagonists and Ang II were dissolved in 0.9% w/v saline. At the beginning of reperfusion, the antagonists were administrated as a bolus dose of 50 µg/kg followed by continuous infusions at 50 µg/kg/h for A779 and bolus dose of 1 mg/kg followed by continuous infusions at 1 mg/kg/h for PD123319 using microsyringe pumps (New Era Pump System Inc. Farmingdale, NY, USA). The antagonists or vehicle was infused via jugular vein as soon as reperfusion was begun and continued during the experiment.

#### Angiotensin II infusion

Thirty min post commencing antagonist infusion, Ang II (0, 100, and 300 ng/kg/min) was administrated using a microsyringe pump. Each dose of Ang II was administered for a 15 min period, and the last 3-5 min was considered for measurements. Originally, the Ang II is rapidly cleared from the circulation (the half-life is 13 s), therefore the Ang II was administrated continually from low to high doses. During Ang II infusion, RPP was maintained at pre-Ang II infusion levels through manipulation of the aortic clamp. MAP, and RBF responses were determined, and RVR was calculated by RPP/RBF ratio.

The rats were sacrificed at the end of experiment, and the left kidney was rapidly removed and weighed to correct the parameters for kidney weight.

#### Statistical analysis

Data are expressed as mean ± SEM and were analyzed using the statistical software SPSS version 20. The repeated measure ANOVA followed by LSD post hoc test (for MAP, RPP, and RBF from Ang II 0-300 ng/kg/min) was used to compare the effect of each treatment using the factors treatment (*P* group), Ang II (*P* dose), and the interaction between treatment and Ang II (*P* group × dose). Statistically *P* < 0.05 was considered significant.

## RESULTS

#### Baseline measurements and response to antagonists

Table 1 shows the basal measurements as control for MAP, RPP, and RBF corrected for kidney weight. During the moderate ischemia (hypo-perfusion), MAP was increased, and as expected, RPP and RBF were decreased, however, these alterations were similar between the groups and no significant differences between the groups for MAP, RPP, and RBF in both male and female were observed (Table 1).

**Table 1.** Data for mean arterial pressure (MAP), renal perfusion pressure (RPP), and renal blood flow per left kidney wet weight (RBF/KW). Data are presented as mean ± SEM and were analyzed using a repeated-measures ANOVA. B, from before ischemia; I, during moderate ischemia; and A, 30 min after reperfusion using the time (*P* time) group treatment (*P* group) and the interaction between time and group treatment (*P* time × group); n = 6 to 8 per group.

Measured factors	Groups	Gender					
		Different time in males			Different time in females		
		B	I	A	B	I	A
MAP (mmHg)	Vehicle	104.7 ± 4.1	125.2 ± 6.2	103.3 ± 4.7	101.9 ± 1.0	119.9 ± 4.2	108.1 ± 1.9
	A779	105.9 ± 0.9	130.4 ± 2.8	107.4 ± 3.4	103.9 ± 1.8	126.0 ± 2.1	107.6 ± 2.2
	A779 + PD123319	103.3 ± 1.4	129.8 ± 4.9	106.9 ± 2.9	101.5 ± 1.6	119.2 ± 3.6	107.7 ± 1.5
	Analysis	$P_{\text{time}} < 0.0001, P_{\text{group}} = 0.64, P_{\text{time} \times \text{group}} = 0.87$			$P_{\text{time}} < 0.0001, P_{\text{group}} = 0.51, P_{\text{time} \times \text{group}} = 0.32$		
RPP (mmHg)	Vehicle	94.8 ± 4.5	24.8 ± 0.8	95.1 ± 4.4	87.4 ± 1.9	23.9 ± 0.9	99.0 ± 2.4
	A779	96.4 ± 2.4	24.6 ± 0.9	101.9 ± 4.5	92.7 ± 2.5	23.3 ± 0.7	97.8 ± 3.5
	A779 + PD123319	92.8 ± 2.0	23.2 ± 0.8	101.1 ± 3.5	88.5 ± 2.5	23.1 ± 0.5	98.8 ± 2.1
	Analysis	$P_{\text{time}} < 0.0001, P_{\text{group}} = 0.58, P_{\text{time} \times \text{group}} = 0.42$			$P_{\text{time}} < 0.0001, P_{\text{group}} = 0.83, P_{\text{time} \times \text{group}} = 0.37$		
RBF/KW (mL/min.g tissue)	Vehicle	2.73 ± 0.34	0.65 ± 0.04	2.82 ± 0.35	3.28 ± 0.32	0.90 ± 0.08	3.62 ± 0.38
	A779	3.26 ± 0.24	0.83 ± 0.06	3.0 ± 0.20	3.0 ± 0.22	0.80 ± 0.06	2.91 ± 0.21
	A779 + PD123319	2.83 ± 0.25	0.70 ± 0.05	2.58 ± 0.25	3.06 ± 0.23	0.96 ± 0.06	2.82 ± 0.23
	Analysis	$P_{\text{time}} < 0.0001, P_{\text{group}} = 0.51, P_{\text{time} \times \text{group}} = 0.65$			$P_{\text{time}} < 0.0001, P_{\text{group}} = 0.39, P_{\text{time} \times \text{group}} = 0.043$		

A77, MasR antagonist; PD123319, AT2R antagonist.

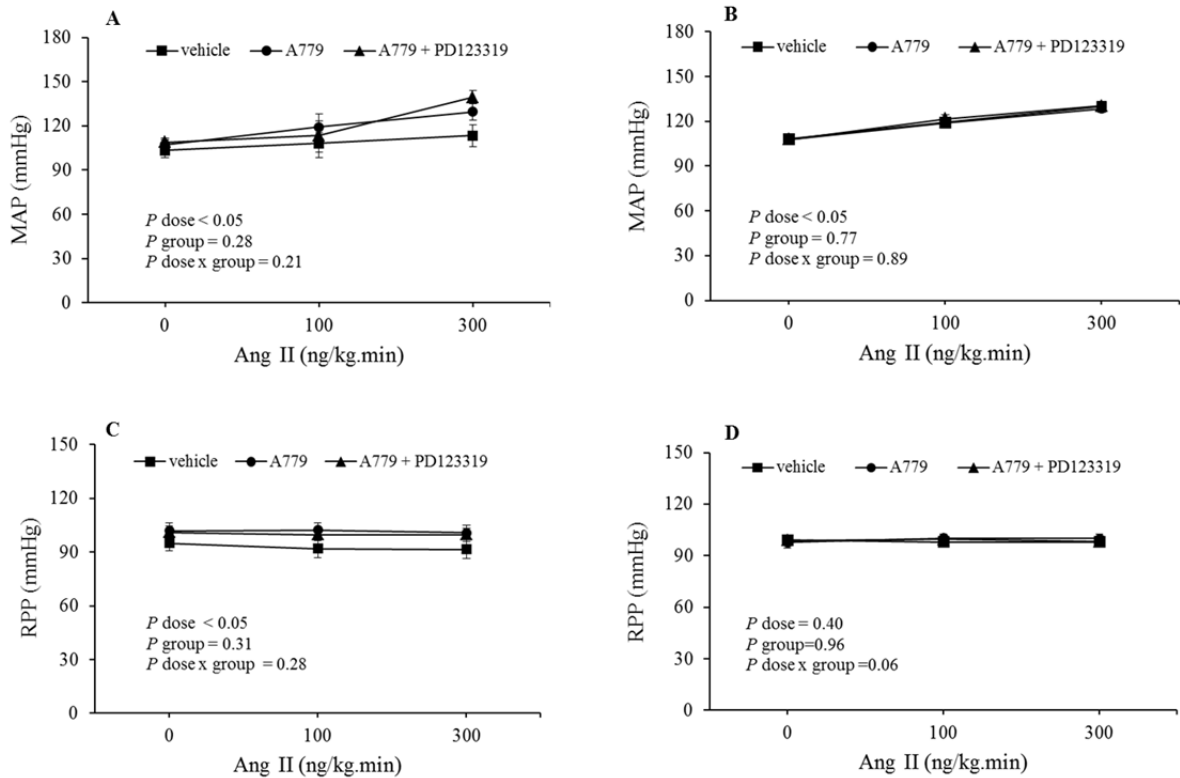
**Responses to angiotensin II infusion**

Ang II infusion increased MAP significantly in a dose-related manner similarly in the vehicle and antagonist treated male and female rats, and as mentioned before, RPP was kept relatively constant during Ang II infusion by the manipulation of the aortic clamp (Fig. 1). However statistical analysis indicated no significant difference for MAP and RPP responses to Ang II infusion in both male ( $P = 0.64$ ) and female ( $P = 0.51$ ) between the groups.

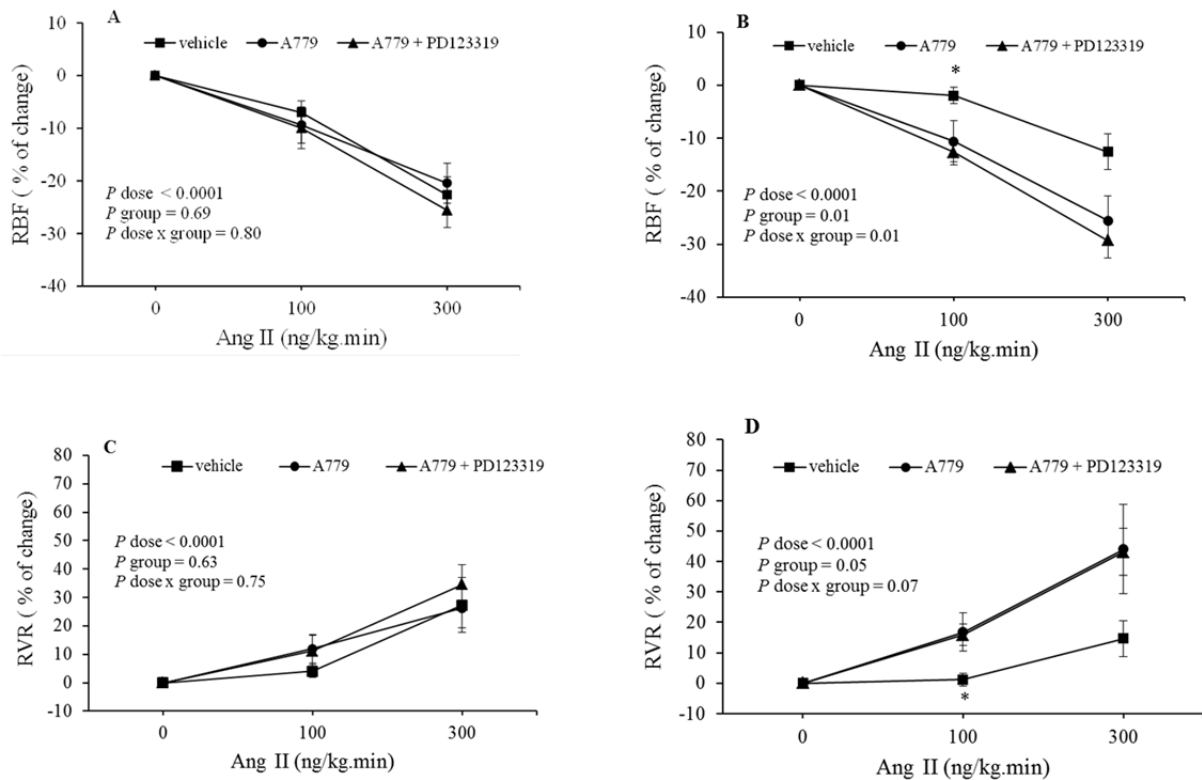
The percentage changes of RBF in response to Ang II infusion are shown in Fig. 2. RBF

decreased and RVR increased in a dose-related manner in response to Ang II infusion ( $P < 0.0001$ ) in both genders (Fig. 2). In male rats, no statistical difference was detected between the groups in RBF and RVR responses to Ang II infusion, however in female rats, A779 alone or A779 plus PD123319 increased RBF and RVR responses to Ang II infusion when compared with vehicle-treated group ( $P < 0.05$ ).

In addition, when PD123319 was accompanied with A779, no additive responses in RBF and RVR to Ang II compared to A779 alone was observed.



**Fig. 1.** Measurements of mean arterial pressure (MAP), renal perfusion pressure (RPP) in response to angiotensin II (Ang II) (0, 100, 300 ng/kg.min) administration in six male (A and C) and female (B and D) experimental groups. ANOVA for repeated measures indicated no significant differences between the groups. Vehicle groups received saline + Ang II, A779 groups received A779 + Ang II, and A779 + PD123319 groups received A779 + PD123319 + Ang II. A779, MasR antagonist; PD123319, AT2R antagonist.



**Fig. 2.** Percentage change of renal blood flow (RBF) (% of change) and percentage change of renal vascular resistance (RVR) (% of change) responses to angiotensin II (Ang II) (0, 100, 300ng/kg.min) administration in six (A and C) and female (B and D) experimental groups. ANOVA for repeated measures indicated significant differences between vehicle group and the other groups in female rats ( $*P < 0.05$ ). Vehicle groups received saline + Ang II, A779 groups received A779 + Ang II, and A779 + PD123319 groups received A779 + PD123319 + Ang II. A779, MasR antagonist; PD123319, AT2R antagonist.

## DISCUSSION

The major findings of this study revealed the impact of gender in renal vascular responses to Ang II infusion when using MasR blockade in renal moderate I/R injury. I/R injury and its outcomes are gendered dimorphic, but a detailed mechanism of this gender-related difference is not well understood (18). However, in our study, the I/R technique had a similar effect on RBF or RPP in both genders. This discrepancy may relate to the model of partial ischemia or related to the interaction between receptors. Kontogiannis *et al.* reported that renal level of Ang II after ischemia may increase by the down-regulation of cortical angiotensinogen and proximal tubular AT1R (19). Also it is well documented that the RAS depressor pathways are more efficient in females than males (20,21), therefore, additional effect is expected when both AT2R and MasR were

blocked simultaneously, however, different response to Ang II was detected when both AT2R and MasR were antagonized (14). MasR's gene is localized on chromosome 6 (22), and in rodents, MasR protein expression found in different segments of the nephron such as the juxtaglomerular apparatus, renal cortical and proximal tubular cells, tubular epithelium, collecting ducts as well as renal vascular parts, that supports its impact in the regulation of renal function (23,24). In humans, MasR expression has been detected in the proximal tubular and mesangial cells (25). MasR as specific receptor of Ang1-7 and its antagonist (A779) may not alter blood pressure in either male or female (26) as confirmed by our study. In the current study, after renal moderate I/R injury, the RBF and RVR responses to Ang II were not different between the male groups (group 1-3) indicating that blockade of AT2R and MasR simultaneously or MasR alone could not significantly alter the

vascular responses to Ang II infusion. Such observation in normal rat models was previously reported (13,14). However, in female rats the co-blockade of AT2R and MasR or MasR alone increased RBF and RVR responses to Ang II significantly and these responses indicated no additive response by PD123319 when it was accompanied with A779. In our previous study in renal moderate I/R model, AT2R blockade (PD123319) significantly and gender dependently increased the renal vascular responses to Ang II infusion (15).

It is documented that Ang II can bind to the AT2R as the same affinity as to the AT1R (27) while AT2R has high affinity for PD123319 (28). In rat kidney, the AT2R mRNA is distinguished from various tubular and vascular parts of the cortex and medulla, including the glomeruli, proximal tubule, collecting duct, arcuate arteries, afferent arterioles, and outer medullary descending vasa recta (29). The other researchers reported that AT2R modulates renal function gender dependently and highlighted the vasorelaxant effect of AT2R in female (15,30). Indeed nitric oxide production and also activation or releasing of bradykinin is involved in vasorelaxant actions of the AT2R. Also predominantly, in women, endothelium-derived hyperpolarizing factor is also involved in the AT2R vasodilator effect (31) and it increases in I/R (32). AT2R vasorelaxant action requires XX chromosome sex complement (31). Research suggested the ability of AT2R blockade for increasing AT1R-mediated effects of Ang II (13).

Renal MasR expression is greater in female compared to male (33) in the way it is reported that the gender difference was abolished in response of arterial pressure to chronic Ang II infusion in angiotensin converting enzyme 2 (ACE2) knockout mice (34) or by chronic MasR blockade (26). Both MasR and AT2R activities are known as vasodilatory responses, so we had expected, though not occurred, a greater RBF response to Ang II during co-administration of AT2R and MasR antagonists. Previously, Safari *et al.* pointed to a paradoxical finding that may be due to cross-talk between the AT1R, AT2R, MasR,

and bradykinin receptor (14). This cross-talk may form heterodimers and *via* constitutive activation regulate receptors expression and activity (35,36). Also G-protein coupled receptors, AT2R, and MasR may interact via the poorly defined signal transduction pathways (37). Previously we observed that PD123319 augmented the Ang II-induced renal vasoconstriction (about 22%) in female I/R rat model (15) just as it reported for intact rats (13), but in I/R rat model combined inactivation of the AT2R and MasR reduced AngII-induced renal vasoconstriction (about 16%) that had been augmented by PD123319 alone in females. This supports other researcher's findings as complex interactions between components of the RAS (35-37).

## CONCLUSION

The AT2R and MasR regulated renal hemodynamic in renal moderate I/R rat model gender dependently. However, unexpected and different response during dual AT2R and MasR blockade between male and female reveals the impact of gender on renal vascular response to Ang II after moderate ischemia

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