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Original Article

The effect of AT_2 and Mas receptors antagonists on renal hemodynamic and excretory disorders induced by ischemia/reperfusion in male and female rats

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Abstract

Introduction: Renal ischemia-reperfusion (RIR) may disturb renin-angiotensin system components. In this study, the effects of Mas receptor (A779) and AT_2 receptor (PD123319) antagonists were examined in RIR rats.

Methods: Total 60 male and female Wistar rats were assigned into 10 groups (n=6 in each group), including sham-operated group, RIR groups treated with the vehicle, A779, PD123319, or A779+PD123319. The rats were subjected to 30 minutes renal ischemia followed by 75 minutes reperfusion and the vehicle/antagonists were started to infuse 15 minutes after beginning of reperfusion for 60 min. Mean arterial pressure (MAP) and renal perfusion pressure responses to antagonists were assessed. Measurements for kidney function parameters also were performed. All the measurements were made at the end of 60 min vehicle/antagonist infusion.

Results: MAP has altered significantly during RIR times (P=0.004), but no significant difference was observed between two genders. The RIR itself in injured rats (compared to sham operated rats) decreased urine flow (UF), creatinine clearance (Ccr), filtrate load of sodium (FNa) and sodium excretion rate (ENa) significantly in both genders (P<0.05). The antagonists infusion caused significant decrease in Ccr and FNa in male and female rats subjected to RIR when compared with vehicle (P<0.05), but the UF decreased significantly (P<0.05) only in PD123319 treated groups; however, there was no significant difference in ENa between the RIR groups in both genders.

Conclusion: Our findings showed the importance role of Mas receptor and AT_2 receptor on renal function after kidney ischemia/reperfusion in RIR rat model.

Keywords:
Renal ischemia-reperfusion;
Mas receptor;
AT_2 receptor;
Renal Function;
Gender

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Introduction

Renal ischemia-reperfusion (RIR) is characterized by a transient decreasing of renal blood flow followed by restoration and reoxygenation (Bazzano et al., 2015). It is recognized as a major cause of acute renal failure which leads to high morbidity and mortality rate (Bonventre and Yang, 2011). Renin-angiotensin system (RAS) and gender alter the renal injury

following RIR (Kontogiannis and Burns, 1998) while RAS has been considered as an important regulatory system of renal function (Jalal Hassanshahi et al., 2017; Rice et al., 2004). Furthermore, female is more resistant than male to RIR induced kidney injury (Fekete et al., 2004) and the RAS functions are different between two genders (Hilliard et al., 2011). Moreover, it has been made clear that the renal function reduces slowly more in female subjected to RIR than male rats (Neugarten et al., 2000; Silbiger and Neugarten, 1995). Generally RAS has two pivotal opposite roles in vascular system. In one hand vasoconstriction by angiotensin II (Ang II) through Ang II type 1 receptor and on the other hand vasodilation by Ang II and angiotensin 1-7 (Ang 1-7) through Ang II type 2 receptor (AT_2R) and Mas receptor (MasR) respectively (Hassanshahi and Nematbakhsh, 2018; Kaschina et al., 2017; Yousif et al., 2017). Hence there are two main RAS vasodepressor pathways (including angiotensin-converting enzyme [ACE]-AT_2R- Ang II and ACE2-MasR-Ang1-7 arms) that regulates the functions of RAS classical pathway (ACE- AT_1 receptor-Ang II arm) (Dilauro and Burns, 2009; Ferrario and Varagic, 2010). In this regard, it is reported that the renal AT_2R expression increased early after RIR and it can attenuate the RIR induced renal injury (Matavelli et al., 2011). Furthermore, it has been shown that Ang 1-7 via MasR has renoprotective effect in RIR model (da Silveira et al., 2010; Maleki et al., 2018) and MasR genetic deficiency can lead to glomerular hyperfiltration and renal fibrosis (Pinheiro et al., 2009). Also, it is known that angiotensin antagonist's administration can exert significant effects on the kidney subjected to RIR (Rabie et al., 2012). According to this result, we hypothesized that in RIR model, AT_2R and MasR can have an important role in modifying the renal injury. In order to assess this hypothesis, renal functions were evaluated while AT_2R and MasR were blocked by PD123319 and A779 respectively in male and female RIR rat models.

Materials and methods

Animal

The total of 60 male and female Wistar rats (male: 202.67±0.84 g, n=30 and female: 178.13±0.83 g, n=30) were housed in a temperature of 23-25°C with a 12 h light/dark cycle and were fed with rat chow and had free access to tap water ad libitum. The maintenance and care of experimental animals comply with National Institutes of Health guidelines for the humane use of laboratory animals, and has been confirmed by the National Institute of Medical Research (NIMAD, # 943759).

Male or female rats were divided into 5 groups. In summary, the designed groups were as follows (n=6 rats in each group): group 1, sham-operated male rats treated with vehicle (saline); group 2, bilateral RIR male rats treated with vehicle (control group); group 3, bilateral RIR male rats treated with MasR antagonist (A779); group 4, bilateral RIR male rats treated with AT_2R antagonist (PD123319) and group 5, bilateral RIR male rats treated with both PD123319 and A779. The groups 6-10 were included female rats that received the same regimen as groups 1-5.

Surgical preparation

Rats were anesthetized with 1.7g kg^{-1} bodyweight urethane (Sigma St. Louis USA). The trachea was isolated to insert an air ventilation tube in order to facilitate breathing. The left jugular vein was cannulated with polyethylene tubing (PE 9658, Microtube Extrusions, North Rocks NSW, Australia) for vehicle/antagonists infusion. Also, catheters were implanted into the left carotid and femoral arteries connected to a pressure transducer and a bridge amplifier (Scientific Concepts, Vic., Melbourne, Australia) for measuring mean arterial pressure (MAP) and renal perfusion pressure (RPP) respectively. MAP and RPP were monitored during the experiment continuously. The bladder also was cannulated to drain urine during the experiment. Finally, under general anesthesia, surgery was performed through an incision on left and right quadrant of the abdomen under sterile conditions and the renal vessels were prepared to induce ischemia by vessels clamping. During the entire period of surgical procedure rectal temperature was maintained at 37±1°C.

Experimental protocol: baseline measurement and RIR induction

The animals were allowed to stabilize for 30-45 minutes as equilibrium time for baseline measurements. The baseline data for MAP and RPP were obtained over the last 5 minutes of equilibrium

Table 1: Mean arterial pressure (MAP) in equilibrium (control), 30 minutes after renal vessels occlusion (ischemia) and 15 minutes post reperfusion (reperfusion). The *P* values were obtained by ANOVA for repeated measures data.

MAP (mmHg)	Male (n=30)	Female (n=30)
Control	102.68±0.99	101.66±0.88
Ischemia	105.57±2.37*	104.58±2.50*
Reperfusion	100.01±2.75	99.27±1.89
$P_{\text{Repeated measure}}$	*P*time = 0.004, *P*gender = 0.68, *P*time x gender = 0.99	

*Ischemia did not apply to sham operated rats, but the data is included.

time. Then in RIR groups, the left and right renal arteries were ligated with micro bulldog clamp and kidneys were subjected to ischemia for 30 minutes and they followed by reperfusion for 75 minutes. MAP and RPP were monitored during the RIR period and data for MAP and RPP were obtained over the last 5 minutes of 30 min renal ischemia time and between 10 and 15 minutes after the reperfusion period as reperfusion time. The experimental protocol was done in all animals but in sham-operated groups was done without RIR induction.

ntagonist responses

Based on groups specified, the animals were subjected to receive either vehicle (saline), MasR antagonist (A779, Bachem, King of Prussia, MO, USA) or AT_2R antagonist (PD123319, Sigma, St. Louis, MO, USA). The A779 and PD123319 were dissolved in 0.9% w/v saline and at the 15 minutes after the beginning of reperfusion the vehicle or antagonist were infused. A779 was administered via jugular vein tube as a bolus dose of 50 µg kg^{-1} followed by continuous infusion at 50µg kg^{-1} h^{-1} using microsyringe pumps (New Era Pump System Inc. Farmingdale, NY, USA). PD123319 was administered with bolus doses of 1 mg kg^{-1} followed by continuous infusion at 1 mg kg^{-1} h^{-1} using microsyringe pumps. The 60 minutes post vehicle or antagonist infusion was considered as antagonist effect time for the measurement. MAP and RPP were determined over the last 5 minutes period of antagonists' effect time. Urine sample also was obtained after the 60 min of vehicle/antagonist infusions. Finally, blood samples were obtained via heart puncture and the animal was sacrificed humanly. Then the serum and urine creatinine (Cr) levels were determined by commercial kits (Pars Azmoon, Tehran, Iran) using RA-1000 system. Also, the serum and urine Na^+ levels were obtained using flame photometer assay.

Statistical analysis

Data analyzing was done by SPSS 20 software and presented as the mean±SEM. ANOVA for repeated measure data and two ways ANOVA were applied using Tukey test as post hoc test. $P \leq 0.05$ was considered to be significant.

Results

Baseline and ischemia-reperfusion measurements

The results showed that there was no significant difference in terms of MAP and RPP between male and female groups in equilibrium period (Table 1), but during renal ischemia, MAP increased. Instead, 15 minutes post-reperfusion, MAP decreased toward normal level and no significant difference was detected between male and female groups in the MAP in ischemia and reperfusion times (Table 1).

Effect of ischemia/reperfusion

The 15 min after beginning of reperfusion, the vehicle was infused for period of 60 min in the sham operated groups (groups 1 and 6) and in the RIR induced groups (groups 2 and 7). The results indicated that vehicle (saline) has no significant effect on MAP and RPP between sham-operated rats (male and female; groups 1 and 6) and RIR rats (male and female; groups 2 and 7) (Fig. 1). However, urine flow (UF), creatinine clearance (Ccr) , filtrate load of Na (FNa) and Na-excretion rate (ENa) decreased significantly in the RIR rats treated with vehicle rather than sham-operated rats treated with the vehicle in each of genders ($P<0.05$, Fig. 1); however there was no significant difference between two genders. Moreover, ENa was significantly ($P<0.05$) different between genders in the RIR rats treated with vehicle (Fig. 1).

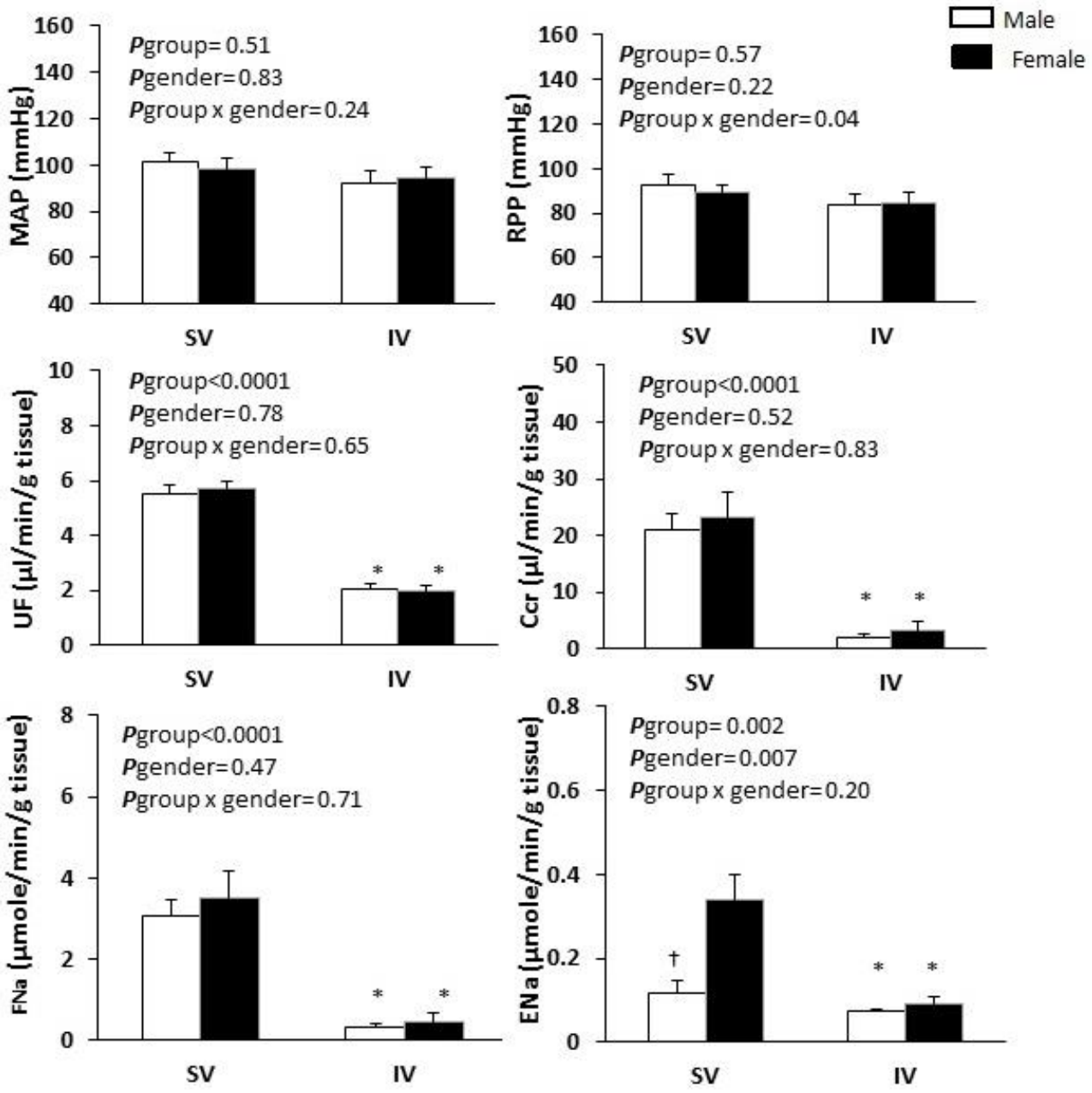

Fig.1. The effect of RIR on mean arterial pressure (MAP), renal perfusion pressure (RPP), urine flow (UF), creatinine clearance (Ccr), sodium excretion rate (ENa) and filtrate load of sodium (FNa) in sham operated animals treated with vehicle (SV) and RIR animals treated with vehicle (IV) after 60 minute post vehicle infusion. Data are presented as mean±SEM. The *P* values were derived from two ways ANOVA. Specific contrasts were generated by Tukey test comparisons. n=6 in each group. * Represents significant difference from SV in the same gender ($P< 0.05$). † Represents significant difference from female rat in similar group ($P< 0.05$).

The serum levels of Cr were 0.59±0.1, 0.8±0.03, 0.64±0.05 and 0.48±0.06 mg/dl in groups 1, 2, 6 and 7 respectively (P_{group} = 0.67, P_{gender} = 0.07, $P_{group\ x\ gender}$ = 0.01). The serum levels of Na also were 146.3±1.9, 153±0.9, 150.3±1.4 and 145.5±3.4 mmole/l in groups 1, 2, 6 and 7 respectively (P_{group} = 0.68, P_{gender} = 0.43, $P_{group\ x\ gender}$ = 0.02); however, no significant differences in Cr and Na levels were detected between the groups and genders.

Effect of antagonists' administration after ischemia/reperfusion

The 15 min after beginning of reperfusion, the vehicle or antagonists were infused for period of 60 min in the RIR groups (groups 2-5 and groups 7-10) and the measurements were compared. The results showed that vehicle or antagonists (A779, PD123319, PD123319+A779) infusion did not have any significant effect on MAP and RPP in both genders

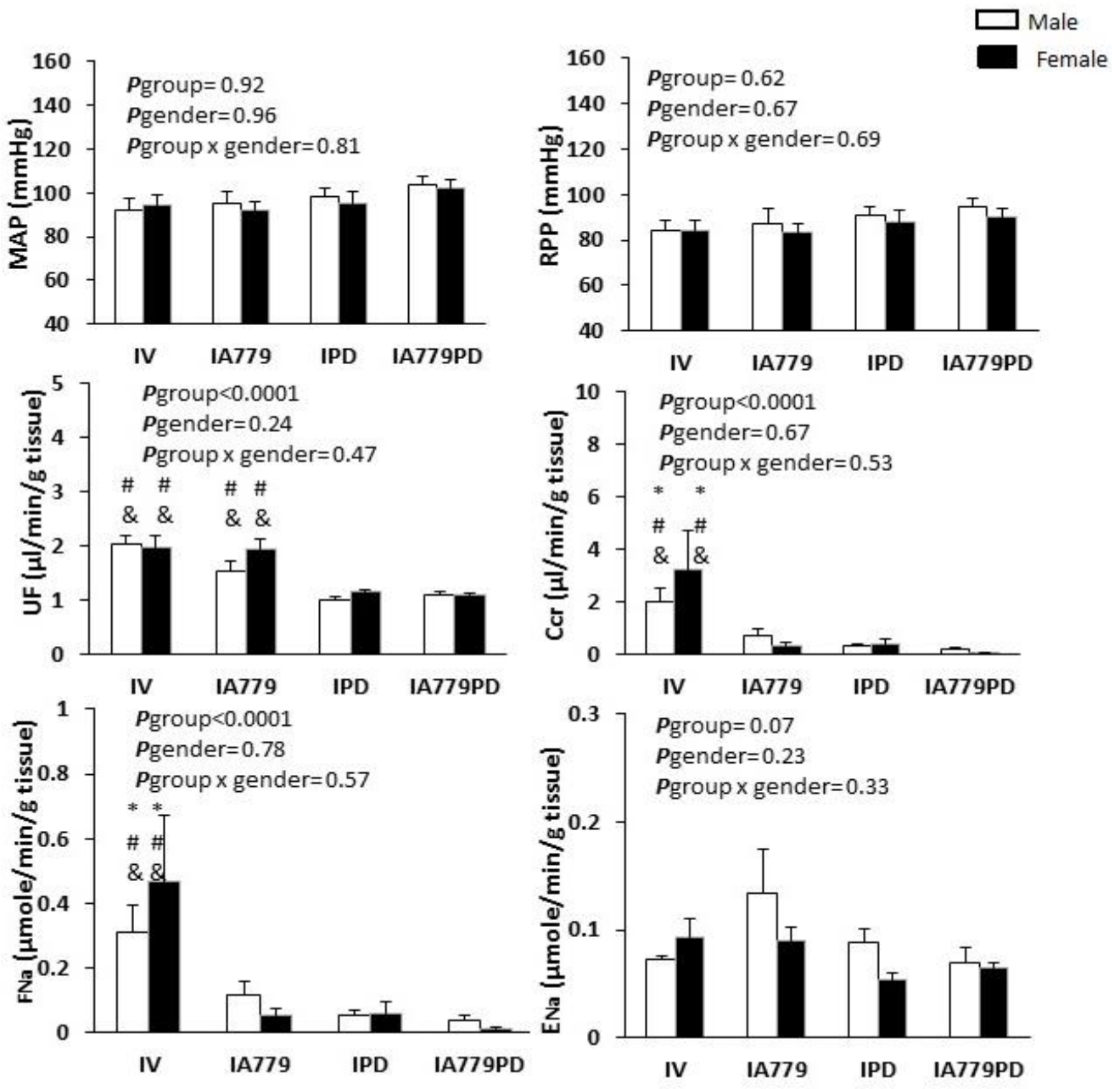

Fig.2. The effect of vehicle (IV), A779 (IA779), PD123319 (IPD) and A779+PD123319 (IA779PD) infusion during 60 minutes post reperfusion on mean arterial pressure (MAP), renal perfusion pressure (RPP), urine flow (UF), creatinine clearance (Ccr), sodium excretion rate (ENa) and filtrate load of sodium (FNa) in RIR animals treated with vehicle (IV)/or antagonists (IA779, IPD, IA779PD). Data are presented as mean±SEM. The *P* values were derived from two ways ANOVA. Specific contrasts were generated by Tukey test comparisons. n=6 in each group. The symbols represents significant difference from (*) A779, (#) PD123319 or (&) A779+PD123319 treat groups ($P< 0.05$).

(Fig. 2); however, UF significantly decreased in both RIR male and female rats treated with PD123319 or PD123319+A779 when compared with vehicle treated rats ($P<0.002$), but no significant difference was detected between two sexes. Our result also showed that Ccr and FNa decreased significantly ($P<0.05$) in the RIR rats treated with A779 or PD123319 or PD123319+A779 compared with the RIR rats treated with vehicle (Fig. 2). However, there were no significant differences in ENa between all RIR groups (Fig. 2).

The serum levels of Cr were 0.8±0.03, 0.66±0.06, 0.65±0.05 and 0.61±0.06 mg/dl in groups 2-5 and 0.48±0.06, 0.7±0.05, 0.61±0.04 and 0.65±0.04 mg/dl in groups 7-10 respectively, while no significant differences were detected between the groups and genders (P_{group} = 0.74, P_{gender} = 0.07, $P_{group\ x\ gender}$ = 0.004). The serum levels of Na also were 153±0.9,

163.5±2, 165.1±1.3 and 172.5±1.8 mmole/l in groups 2-5 and 145.5±3.4, 157.7.7±3.3, 159±1.6 and 157.8±2.2 mg/dl in groups 7-10 respectively, while significant differences were detected between the groups; vehicle treated group was significantly different from others groups, and the difference between the genders was detected (P_{group} <0.0001, P_{gender} <0.0001, $P_{group\ x\ gender}$ = 0.18).

Discussion

The major findings of this study reveal that RIR itself decreased renal functions indicators and MasR and AT2R antagonists reduce it more severely. In addition vehicle or antagonists have no significant effect on MAP and RPP (Figs. 1 and 2). Our previous study has shown that bilateral renal artery ligation increased the systemic arterial pressure (Maleki and Nematbakhsh, 2016). Also, it was shown that RIR only for 10 minutes induce renal tubular injury (Palacio et al., 1997) and Ccr may impair (Schrier et al., 2004). In this regard, our study showed that UF, Ccr and FNa significantly decreased in the RIR vehicle treated rats compared with the sham-vehicle treated rats (Fig. 1) and no significant difference was detected between two genders. In line with our study, it has been shown that the bilateral RIR decreases the glomerular filtration rate which is characterized by an increase in serum Cr level (Barroso et al., 2012). Moreover, serum osmolality increased and urinary osmolality decreased in RIR model (Barroso et al., 2012). These changes participate in renal dysfunction and kidney injury through increasing the osmotic active molecules levels, while urinary concentration ability decreased possibly because of renal tubules injury (Barroso et al., 2012). Collectively, these renal functional impairments confirm an acute kidney injury after ischemia/reperfusion (Abuelo, 2007; Bellomo et al., 2004). Our study also showed that UF significantly decreased without gender difference in the RIR groups treated with PD123319 or PD123319 plus A779 when compared with the vehicle treated rats (Fig. 2). In this regard, it has determined that reactivation of AT_2R can be done in renal proximal tubules after RIR (Zhang et al., 2004), but it is PD123319 sensitive (De Souza et al., 2004). Also diuretic effect of Ang 1-7 mediated via MasR could be blocked by A779 (Santos et al., 2013). The current study also indicated that Ccr and FNa significantly decreased in male and female rats subjected to RIR received antagonists (A779 or PD123319 or PD123319 plus A779) rather than vehicle (Fig. 2). It has been shown that RIR can alter the RAS component's activity, especially receptors numbers and local angiotensin's level in kidney tissue (Santos et al., 2008); for example, it has been reported that reperfusion for 4 hours increases the kidney Ang II levels and decreases the Ang 1-7 in the RIR model (da Silveira et al., 2010). Moreover, it has been shown that the MasR protein expression increases in renal cortex and medulla tissues in RIR model (da Silveira et al., 2010). Also, AT_2R had kidney protective effect in female rat subjected to RIR model (Maleki and Nematbakhsh, 2016). Moreover, it has been seen that the level of renal AngII was two times higher, but the renal Ang1-7 level was three times lower in the RIR male rats while compares to the RIR female rats (Chappell et al., 2014). It seems that A779 or PD123319 inhibits the vasodilatory effect of AngII and Ang1-7 on renal circulation and FNa and Ccr were reduced. It also has been shown that sex hormones have a protective role in RIR model (Hutchens et al., 2008; Park et al., 2004;), although the exact mechanisms are not specified (Hutchens et al., 2012). In another view, it has been made clear that in both males and females normal rats, AT_2R antagonist decreases the natriuresis (Gross et al., 2000) and also UF and ENa alters gender dependently when RPP rises (Hilliard et al., 2011). In addition, on one hand, it is specified that the renal AT_2R expression is greater in female rather than male rats and estrogen as a sex hormone can up-regulate the AT_2R expression in female rats, and on the other hand, the new AT_2R expression has been observed in different parts of renal tubule and glomeruli in the kidneys with RIR (Kontogiannis and Burns, 1998). Our study showed that if the RAS vasodilator arms receptors (MasR or AT_2R) were blocked, the renal functional indicators (UF, FNa and Ccr) were decreased in the RIR rats compared with the sham-operated groups. Moreover, the AT_2R vasodilator properties can be caused via endothelium-derived hyperpolarizing factors (Danser et al., 2015) and also these factors are higher in female than male rats and increased in the RIR model (Marrelli, 2002). Collectively, the renal functional responses to AT_2R and MasR antagonists' administration could be due to the role of these receptors in renal circulation after

RIR.

Conclusion

According to our findings, it seems that the renal functional responses to MasR and AT2R antagonists are almost disturbed, but are not completely gender dependent in rats subjected to RIR. The therapeutic look at RAS vasodepressor pathway receptors (AT2R and MasR) may be useful following RIR condition in the future.

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Conflict of interest

The authors declare no conflict of interests.

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