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Experimental Research Article

The role of the AT1 receptor antagonist on renal hemodynamic responses to angiotensin 1-7 in acute sympathectomized male and female rats

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ABSTRACT

Introduction: The sympathetic nervous system and the renin-angiotensin system (RAS) are the most pivotal vasoactive systems in regulating renal hemodynamics. The main objective of this study was to determine the role of the angiotensin II (Ang II) type 1 receptor (AT1R) antagonist on renal hemodynamic responses to Ang 1-7 infusion in innervated and denervated male and female rats.

Methods: Male and female Wistar rats underwent unilateral nephrectomy. Four weeks later, they were divided into two groups: innervated and acutely denervated groups. Subsequently, the anesthetized and catheterized rats in both groups were treated with saline as a vehicle and losartan infusion. Mean arterial pressure (MAP), renal blood flow (RBF), renal perfusion pressure (RPP), and renal vascular resistance (RVR) responses to Ang 1-7 (100, 300, and 1000 ng kg−1 min−1) were then measured at controlled RPP.

Results: Basal MAP, RPP, RBF, and RVR did not show significant differences between the intact and denervated groups. Losartan significantly decreased MAP, RPP, and RVR in both innervated and denervated male and female rats ($P<0.001$), while RBF increased only in innervated and denervated female rats ($P<0.004$). However, following Ang 1-7 administration, the RBF response to Ang 1-7 infusion differed significantly between intact and denervated male rats treated with losartan ($P<0.04$). This response was not observed in female rats.

Conclusion: These data suggest a synergistic effect of losartan and Ang 1-7 on increased RBF in the presence of renal sympathetic nerves in male rats.

Keywords:
Angiotensin 1-7
Sympathectomy
Losartan
Renal blood flow

Introduction

Absolutely, the sympathetic nervous system (SNS) plays a crucial role in regulating renal function and blood pressure. By modulating renal hemodynamics, tubular reabsorption, and renin secretion, the renal nerves help maintain volume and sodium balance and regulate renin release. (Schlaich et al., 2012). It is known that the basal activity of the sympathetic nerves in the kidney is not

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significant under normal conditions. However, in conditions such as hypertension, the activity of these nerves increases (Abdulla et al., 2009; Katsurada et al., 2022; Veiga et al., 2021). Regarding the heightened activity of renal sympathetic nerves in conditions like hypertension and other related kidney disorders, one therapeutic strategy employed is renal denervation (RDN) (Kassab et al., 2022; Scalise et al., 2020). However, conflicting findings exist regarding renal hemodynamics during renal denervation (Kazi 2010). Due to regional differences (Kacem and Sercombe 2006) and gender differences (Caplea et al., 2002; Joyner et al., 2015; Sandberg et al., 2015), sympathetic denervation in different vascular beds and sexes influences the neural control of the systemic and local circulations differently.

The renin-angiotensin system (RAS) is another pivotal vasoactive system involved in regulating renal hemodynamics and renal sodium reabsorption under both physiological and pathological conditions (Urushihara and Kagami 2017). The RAS is involved in maintaining renal hemodynamics through the balance between its vasoconstrictor and vasodilator arms (Kobori et al., 2007; Unger 2002). The vasoconstrictive function of the RAS is mediated by angiotensin II (Ang II) and its two receptor types, AT1R and AT2R (Carey 2015). Ang II, acting via the AT1R receptor, participates in cell growth and differentiation, regulation of blood pressure, and modulation of sympathetic neurotransmitter release (Fountain et al., 2023; Stegbauer et al., 2003). This receptor can be blocked by losartan (Sun et al., 2012). The vasodilatory function of RAS is mediated by angiotensin 1-7 (Ang 1-7) peptide, which in kidney tissue is formed from the hydrolysis of Ang II or Ang I by angiotensin-converting enzyme 2 (ACE2) (Povlsen et al., 2020; Velez et al., 2009). The Mas receptor is responsible for most of its effects in a sex-dependent manner (Santos et al., 2003). Many investigations have shown the intricacy of renal functions of Ang 1-7 (Carey and Siragy 2003; Chappell et al., 2004; e Silva et al., 2006; Ferrario and Chappell 2004). Depending on the diameter of the vessels, the local vascular beds, and the species, different mechanisms are proposed for Ang 1-7 vasodilatory effects (Santos et al., 2013). It can also interact with AT1R, AT2R, bradykinin (BK) B2 receptors (B2R) (De Moura et al., 2004; Dilauro and Burns 2009; Magaldi et al., 2003; Oudit et al., 2007). This indicates complex interplays between RAS receptors and the vascular function of Ang 1-7 (Pinheiro and Simões e Silva 2012). In addition, gender differences have been identified in the expression rates of RAS components and responses to RAS excitation and inhibition under physiological and pathophysiological conditions (Komukai et al., 2010; Sampson et al., 2008; Sullivan 2008; Xue et al., 2007).

Given that the activity of the RAS and the sympathetic nervous system affects kidney hemodynamics, understanding the interactions between renal sympathetic nerves and RAS in the control of renal function is very important (Kopp and DiBona 2020). Targeting the ACE2/Ang(1-7)/MasR axis is an emerging strategy to modulate the functions of the cardiovascular and renal systems, which may show the therapeutic potential of these peptides (Liao and Wu 2021; Medina et al., 2020). Increasing evidence suggests the anti-inflammatory effect of the ACE2/Ang(1-7)/MasR axis as a depressor arm in the RAS, to balance the effect of the Ang II/AT1R axis (Simões e Silva et al., 2013; Zhang et al., 2014). Therefore, the question arises, what is the role of the depressor arm of RAS in kidney circulation in the presence or absence of the sympathetic system? Is there interference between the receptors of the RAS in the presence or absence of the sympathetic system? The answer to this question is important in clinical conditions such as kidney transplantation (in terms of acute sympathectomy in the early stages) to control renal hemodynamics. Also, the related changes in renal function and hemodynamics following renal denervation in normal conditions, as well as the role of Ang 1-7 in adjusting renal hemodynamics, require further recognition since the findings are inconsistent. The present study aimed to investigate the renal vascular responses to Ang 1-7 administration in denervated and innervated rats of both sexes. In addition, the role of AT1R on renal hemodynamic response to Ang 1-7 administration in the presence or absence of renal sympathetic nerves was also determined.

Materials and Methods

Animals

In the present study, 35 male and 35 female Wistar rats aged 7-8 weeks, weighing 150–200 g, were provided by the Animal House of the Water and Electrolyte Research Center of Isfahan University of Medical Sciences. The rats were housed individually in standard cages with a room temperature of 23–25°C and a 12-hour light/dark

TABLE 1: The distinguishing features in each phase of estrous cycle (Ajayi and Akhigbe 2020; Lovick and Zangrossi Jr 2021).

Estrous phase	Cycle length (hours)	cell types	Sexual hormone status
Proestrus	14	Many small nucleated epithelial cells. They appear in clusters or individually and with little or no leucocytes	There are rapid changes in the hormonal profile. High levels of estradiol are observed in the morning, and during the afternoon, there is a surge in the secretion of progesterone, leading to the highest concentration achieved during the cycle.
Estrus	24–48 h	Anucleated keratinized epithelial cells or cornified cells	Secretion of progesterone and estradiol remain at a low stable level throughout estrus.
Metestrus	6-8h	The cornified epithelial cells and low to moderate leukocyte and few epithelial cells.	It is characterized by low levels of estradiol.
Diestrus	48–72h	Many leucocytes with little or no cornified cells	Diestrus and metestrus are characterized by low levels of estradiol.

cycle. They had ad libitum access to water and food pellets. All surgical procedures and animal handling followed the guidelines for the care and use of laboratory animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Animal Ethics Committee at Isfahan University of Medical Sciences (Code #: IR. MUI. MED. REC.1400.025).

Surgical procedures

Unilateral nephrectomy

First, the animals were anesthetized with chloral hydrate (450 mg/kg; ip) and xylazine (10 mg/kg; ip). Right unilateral nephrectomy was performed on animals placed in the flank position. A 1 cm longitudinal incision was made on the right side to expose the kidney. Care was taken to separate the kidney from the surrounding fat and tissues, ensuring not to damage the adjacent adrenal gland. The renal artery, renal vein, and ureter of the right kidney were ligated using a 3.0 silk suture, and then the kidney was removed (Zalups 1991).

Acute unilateral renal denervation

First, the left kidney was exposed by creating a flank incision. The renal artery was separated from the surrounding fascia and the vein. Under a dissecting microscope, the renal nerves were carefully dissected. Subsequently, the renal artery was stained with a chemical solution (10% phenol in 90% ethanol) using a swab for 2-3 minutes to ensure the destruction of any remaining nerves (Cai et al., 2018; Khan et al., 2014).

Preparation of vaginal smear

The estrous cycle in rats typically lasts for four days. Our aim was to use female rats in the same stage of the estrous cycle, specifically the diestrus phase. To determine the stage of the estrous cycle, we employed the vaginal smear technique, which involves observing vaginal cells under a microscope. The estrous cycle consists of four main phases: proestrus, estrus, metestrus, and diestrus. These phases can be distinguished by the presence, absence, or relative abundance of leukocytes, nucleated epithelial cells, and non-nucleated epithelial cells (Table 1). The vaginal smear technique involves introducing a small amount of fluid into the vagina using a pipette and rinsing the cells from the vaginal wall. Approximately 0.2 ml of saline is drawn into the pipette tip. The tip of the pipette is gently inserted into the vaginal opening to a depth of 2-5 mm, and the fluid is then expelled into the vagina. The resulting suspension is slowly drawn into the pipette after gently pressing and releasing the pipette two or three times. By placing a drop of the resulting cell suspension onto a slide and examining it under a microscope, we can determine the stage of the rat's estrous cycle. Diestrus smears predominantly contain leukocytes, along with a variable number of nucleated and non-nucleated epithelial cells (Ajayi and Akhigbe 2020).

Experimental design

The nephrectomized rats were randomly assigned to two main groups: non-renal denervated groups (Non-RDN or intact groups) and acute renal denervated groups (acute RDN groups). Each group was further divided into four subgroups, resulting in a total of eight experimental groups: four groups of males and four groups of females.

Groups 1&2: Intact or non-RDN groups of male (n=10) and female (n=10) rats were treated with vehicle and then received Ang 1-7.

Groups 3&4: Intact or non-RDN groups of male (n=8) and female (n=6) rats were treated with losartan and then received Ang 1-7.

Groups 5&6: Acute RDN or denervated groups of male (n=8) and female (n=10) rats were treated with vehicle and then received Ang 1-7.

Groups 7&8: Acute RDN or denervated groups of male (n=9) and female (n=9) rats were treated with losartan and then received Ang 1-7.

Surgical preparation

Four weeks after nephrectomy, the rats were anesthetized with urethane (1.7 g/kg; Merck, Germany). A polyvinyl chloride (PVC) catheter was inserted into the trachea and fixed to facilitate air ventilation. Polyethylene cannulas (PE 9658, Micro tube Extrusions, North Rocks NSW, Australia) containing heparin (50 IU/ml) in 0.9% saline were placed into the left carotid and left femoral arteries and linked to a pressure transducer and an amplifier (AD Instruments, Australia) connected to a computerized data acquisition system (PowerLab, AD Instrumentation, Sydney, Australia) for continuous monitoring of mean arterial blood pressure (MAP) and renal perfusion pressure (RPP). The jugular vein was cannulated for vehicle and antagonist infusion by two micro- injection pumps (New Era Pump System Inc., Farmingdale, NY, USA) throughout the experiment. The animal was posited on its left flank on the surgical desk, and a lateral incision was made along the median anterior axillary line below the level of the coastal edge. This lateral incision provided access to the left kidney. Then, the renal artery was detached from the surrounding tissues and the adjacent vein. At the same time, renal denervation was performed for acute sympathectomized rat groups. The renal blood flow (RBF) was evaluated using a flowmeter probe (TRANSONIC MA0.7PSB, Flow probe, USA) placed on the isolated renal artery. The probe was attached to a flowmeter (T402, Transonic Systems Inc., Ithaca, NY 14850 USA) linked to PowerLab. A adjustable clamp was placed around the abdominal aorta to stabilize RPP during Ang 1-7 infusion. A period of 30 to 60 minutes was allowed for equilibration before administration of the vehicle or antagonist. During the last 5 minutes of equilibration, MAP, RPP, RBF, and renal vascular resistance (RVR) parameters were measured as baseline data, referred to as the "control" phase. RVR was calculated as the ratio of RPP to RBF.

Experimental protocol

Vehicle/Antagonist infusion

At the end of the control phase, all antagonist-treated groups recieved bolus doses (5mg·kg−1) of losartan (Darou Pakhsh Pharma Co., Tehran, Iran), followed by continious injections (5 mg·kg−1·h−1) throughout the investigation. The vehicle-treated groups followed a similar protocol but received 0.9% saline in equal volume instead of the antagonists during the experiment. Both the vehicle and antagonist were continuously ad-

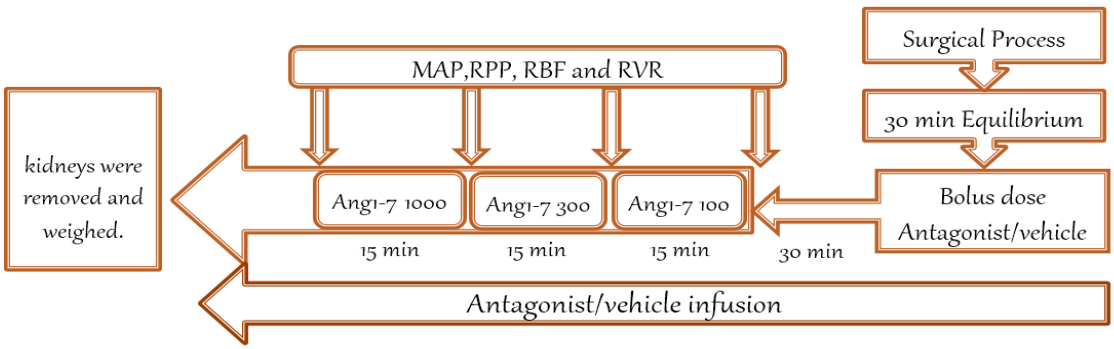

FIGURE 1. The expriment protocol: After the surgical procedures and allowing 30 min for equilibrium, parameters of MAP, RPP, RBF, and RVR were measured in the last 5 min of this period as baseline data. Then, in groups treated with antagonist, the infusion of losartan was conducted at a bolus dose of 5mg/kg followed by injection a continuous of 5 mg/kg/h for 30 min.In groups treated with saline an equal volume of normal saline 0.9% was used instead of losartan as a vehicle. The values of RPP, RVR, MAP, and RBF measured at the final 5 min as treat data. Subsequently, Ang 1-7 was administered for 15 min at doses of 100, 300, and 1000 ng/kg/min, whereas the injection of saline or losartan continued until the end of the experiment. Hemodynamic parameters were measured as a vascular response to Ang 1-7 infusion at the end of each dose as previously described.

TABLE 2: Baseline data for MAP, RPP, RBF, and RVR before the vehicle or antagonist treatment in intact and acute-RDN nephrectomies rats.

	Groups	MAP (mmHg)	RPP (mmHg)	RBF/LKW (ml/min/g lkw)	RVR/LKW (mmHg.min/ml/g lkw)
Treated-saline male	Intact	90.1± 2.9	78.9± 3.7	2.1± 0.2	41.5± 6.1
	Acute	83.1± 2.7	75.2± 3.6	2.7±0.3	29.1± 3.8
	P(t test)	0.11	0.48	0.11	0.13
Treated-losartan male	Intact	93.8±3.9	83.1±3.5	1.8±0.2	45.6±3.2
	Acute	86.8±2.8	81.21±6.8	1.5±0.1	55.6±4.9
	P(t test)	0.16	0.78	0.27	0.13
Treated-saline female	Intact	88.5±3.5	77.8±3.3	2.19±0.17	37.34±3.4
	Acute	80.5±3.5	74.9±4.6	2.15±0.24	37.43±3.9
	P(t test)	0.13	0.61	0.89	0.98
Treated-losartan female	Intact	93.6±4.1	84.3±4.1	1.9±0.16	44.4±5.7
	Acute	84.4±2.2	75.7±3.3	2.25±0.14	34.8± 3.2
	P(t test)	0.08	0.12	0.25	0.15

The results are displayed as mean ± SEM. The statistical P values were concluded using the Unpaired-sample T-test. MAP: mean arterial pressure (mmHg); RPP: renal perfusion pressure (mmHg); RBF/LKW: renal blood flow per gram left kidney weight (ml/min·g) and RVR/LKW: renal vascular resistance per gram left kidney weight (mmHg/ml/min·g)

ministered until the end of the Ang 1-7 infusion (termination of the trial). After 30 minutes of antagonist/vehicle administration, the last 5 minutes of injection were considered the "treat" phase, during which the hemodynamic parameters mentioned in the control phase were measured (Figure 1).

Ang1-7 Infusion

At the end of the antagonist/vehicle phase, Ang 1-7 (Sigma Chemical Company, St. Louis, MO, USA) was perfused at doses of 100, 300, and 1000 ng/kg/min. Each dose was adminstrated for 15 minutes, and hemodynamic parameters were measured during the last 5 minutes of each dose to assess the vascular response to Ang 1-7 administration. Throughout Ang 1-7 infusion, RPP was maintained at pre-Ang 1-7 infusion levels using a regulatable arterial occluder to ensure stable conditions, as any change in RPP can influence the activity of the RAS system via pressure-dependent renin release. Finally, the experiment ended, and the animal was euthanized with an overdose of anesthesia. The left kidney was promptly harvested and weighed (Figure 1).

Statistical analysis

Results are presented as mean ± SEM, and statistical analysis was conducted using SPSS version 22 software. Baseline data between groups were compared using unpaired Student's t-tests. For each gender, differences in MAP, RPP, RVR, and RBF responses (defined as the difference between the control phase and treat phase) were assessed using repeated-measures ANOVA. Additionally, responses to Ang 1-7 were analyzed using repeated-measures ANOVA for the different groups. A P-value ≤ 0.05 was considered statistically significant.

Results

3.1. Baseline data (control phase)

The baseline data within each sex showed no significant variation in MAP, RPP, RBF/left kidney weight (RBF/g LKW), and RVR/left kidney weight (RVR/g LKW) between the innervated and denervated groups before the infusion of vehicle or antagonist (Table 2).

Hemodynamics responses to vehicle/antagonist infusion (treat phase)

MAP, RPP, RBF, and RVR responses to vehicle/antagonist were evaluated in two groups (intact and acute-RDN) of rats treated with saline or losartan in both sexes.

Responses to vehicle infusion

The data from the vehicle phase showed a significant variation in MAP (Pdose= 0.01) within male rat groups treated with the vehicle. However, there was no significant difference in MAP between saline-treated male rat groups. In contrast, there was no significant difference in MAP within or between saline-treated female rat groups. Within female rats treated with the vehicle,

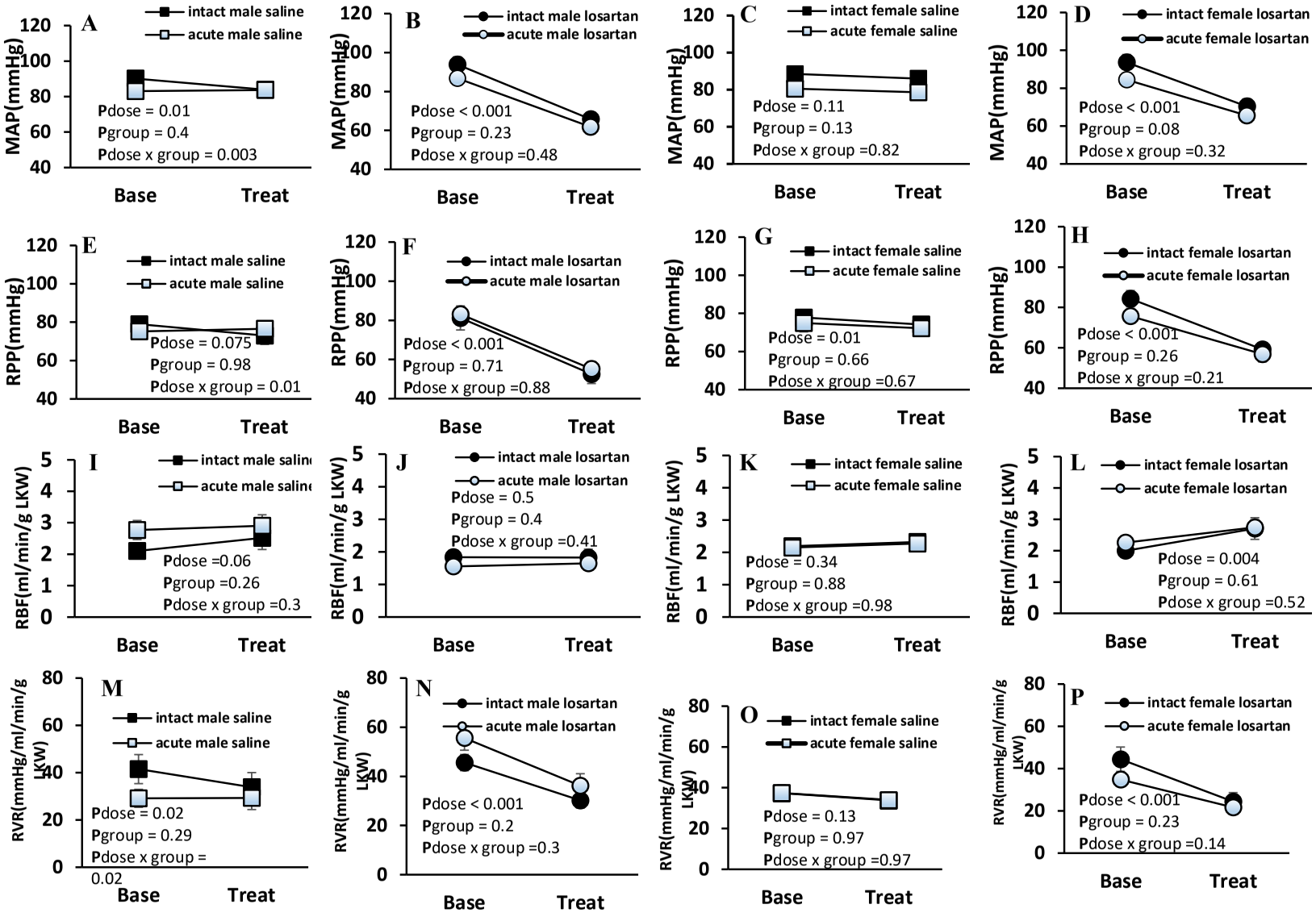

FIGURE 2. The vehicle or antagonist effects: hemodynamic parameters before and after infusion of vehicle or losartan in intact and acutely sympathectomised rats treated with saline or losartan. A, B, C & D: MAP in saline or losartan-treated male and female rats. E, F, G & H: RPP in saline or losartan-treated male and female rats. I, J, K &L: RBF in saline or losartan-treated male and female rats. M, N, O &P: RVR in saline or losartan-treated male and female rats. Results are displayed as the mean ± SEM. MAP: mean arterial pressure (mmHg); RPP: renal perfusion pressure (mmHg); RBF/LKW: renal blood flow per gram left kidney weight (ml/min·g) and RVR/LKW: renal vascular resistance per gram left kidney weight (mmHg/ml/min·g) in all the experimental groups. The P values were obtained by repeated-measures ANOVA, considering the group and their interactions.

there was a significant variation in RPP (P_{dose}= 0.01). However, there was no significant difference in RPP between groups of either gender during saline infusion. There was no significant variation in RBF within male rats treated with the vehicle. However, a significant decrease in RVR (P_{dose}= 0.02) was observed in response to saline infusion in male groups. Conversely, no significant variation in these parameters was observed in female rat groups. Additionally, there was no significant difference in RBF and RVR between groups during vehicle infusion in both sexes (Figure 2).

Responses to antagonist infusion

The data of the antagonist phase demonstrated a significant decline in MAP (P_{dose} < 0.001) in losartan-treated male and female rats compared with baseline levels. Between the losartan-treated male and female subgroups, there was no significant difference in MAP. Similarly, there was a significant decline in RPP (P_{dose} < 0.001) in losartan-treated male and female rat groups compared with baseline. Across all gender groups, there was no significant difference in RPP during losartan infusion. Within male rat groups, there was no significant difference in RBF, while in female rats, there was a tendency towards increased RBF (P_{dose} = 0.004) with losartan infusion compared to the vehicle. However, no significant difference in RBF was observed between female rat groups during losartan infusion. Additionally, there was a significant decline in RVR (P_{dose} < 0.001) in losartan-treated male and female rats compared with baseline. Between groups, there was no significant difference in RVR during losartan infusion in both sexes (Figure 2).

Hemodynamics response to Ang1-7 infusion

MAP, RPP, RBF, and RVR responses to Ang1-7 were evaluated in intact and acute-RDN groups treated with either saline or losartan in both sexes.

Responses to vehicle infusion

There was a significant increase in MAP response to Ang1-7 infusion in groups of saline-treated male rats. A similar trend was observed in saline-treated female rat groups (P_{dose} = 0.02). The vehicle data between the groups of every gender indicated a significant difference in MAP response to Ang1-7 in female groups (P_{group} = 0.03) treated with the vehicle. There was no significant alteration in RPP response to Ang1-7 infusion in groups treated with saline in each sex. As mentioned, RPP was stabilized by manipulating the aortic clamp during the Ang1-7 infusion. Therefore, no variation in RPP was expected to be observed by the Ang1-7 infusion. There was no significant difference in RPP response to Ang1-7 between the groups during the administration of saline in both sexes. There were no significant alterations in RBF and RVR responses to Ang1-7 administration in saline-treated female and male rat groups. In both genders, RBF, and RVR responses to Ang1-7 did not differ between the two groups (Figure 3).

Responses to antagonist infusion

The data of the antagonist phase showed that MAP in response to different doses of Ang1-7 did not change in either losartan-treated male or losartan-treated female rat groups. The antagonist data between groups showed a significant difference in MAP response to Ang1-7 (P_{group} = 0.03) in females but not males treated with losartan. There was a significant difference (P_{dose} = 0.005) in RPP in losartan-treated female rats, although this difference may not be clinically significant. The antagonist data between groups showed no significant difference in RPP between the groups during the administration of antagonists in both sexes. There was a significant increase in RBF response to Ang1-7 infusion (P_{dose} <0.001) within losartan-treated male rat groups. Interestingly, there was a significant difference in RBF response to Ang1-7 (P_{group} = 0.04) in males treated with losartan between the intact and acute-RDN groups during the administration of Ang1-7. In comparison, there was no significant alteration in RBF response to Ang1-7 within and between losartan-treated female rat groups. There was a significant reduction (P_{dose} < 0.001) in RVR response to Ang1-7 administration in losartan-treated male rat groups. The difference in RVR alteration induced by Ang1-7 infusion between the two subgroups was statistically significant (P_{group} = 0.03). There was no significant difference in RVR responses to Ang1-7 within and between losartan-treated female rat groups during the administration of antagonists (Figure 3).

Discussion

This study aimed to assess renal vascular responses to Ang 1-7 administration in innervated and acutely renal denervated female and male rats treated with vehicle or losartan. Our key finding was that losartan

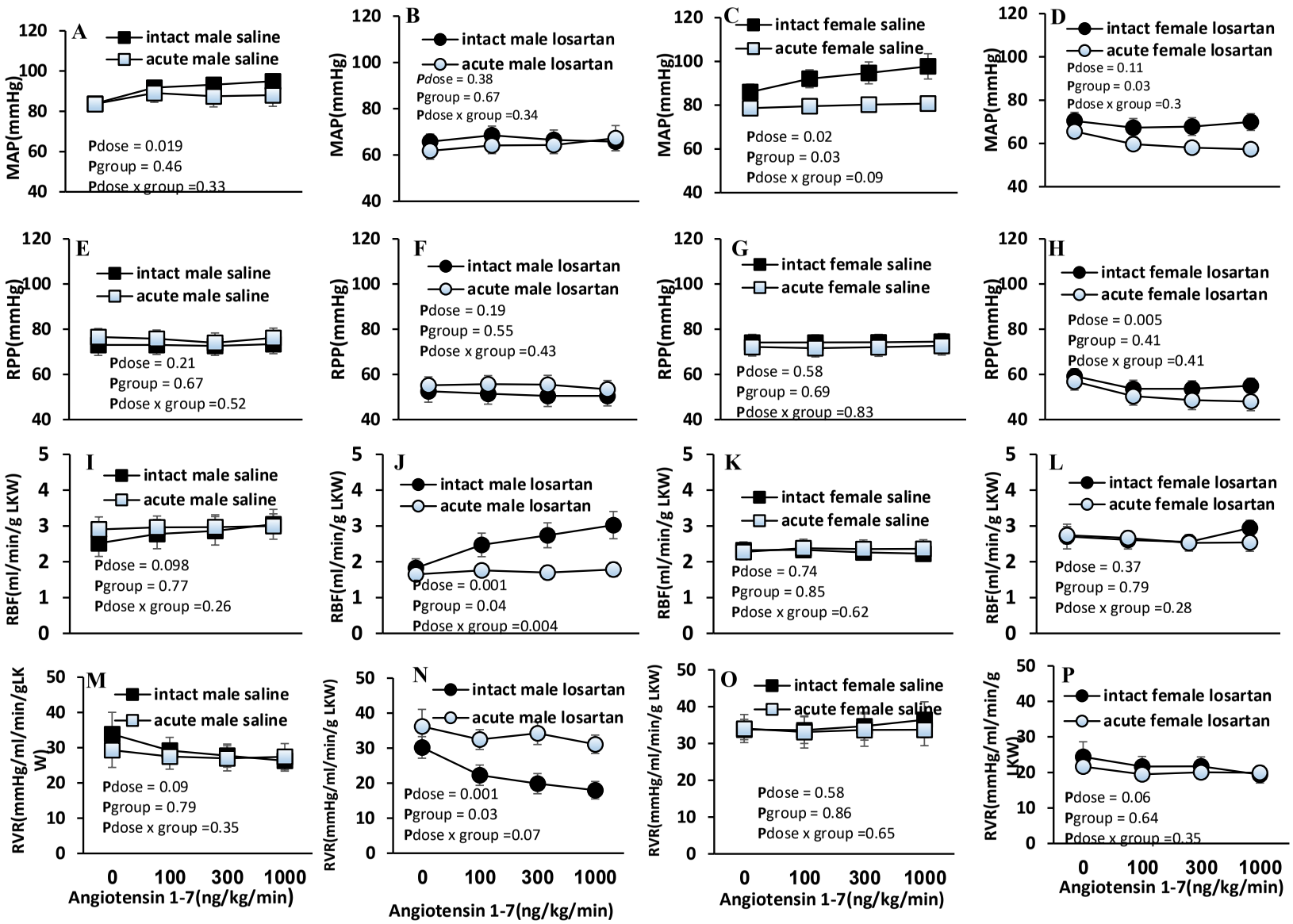

FIGURE 3. The effect of graded angiotensin 1-7 infusion on hemodynamics parameters. After administration of vehicle or losartan in intact and acutely sympathectomised rats treated with vehicle or losartan. A, B, C & D: MAP in saline or losartan-treated male and female rats. E, F, G & H: RPP in saline or losartan-treated male and female rats. I, J, K &L: RBF in saline or losartan-treated male and female rats. M, N, O &P: RVR in saline or losartan-treated male and female rats. Results are illustrated as the mean ± SEM. MAP: mean arterial pressure (mmHg); RPP: renal perfusion pressure (mmHg); RBF/LKW: renal blood flow per gram left kidney weight (ml/min·g) and RVR/LKW: renal vascular resistance per gram left kidney weight (mmHg/ml/min·g) in all the experimental groups. The P values were obtained by repeated-measures ANOVA, considering the group and their interactions.

administration significantly enhanced the RBF response to Ang 1-7 in innervated male rats, with a significantly greater response observed compared to denervated male rats. However, in both innervated and denervated female rats treated with either the vehicle or losartan, no change in RBF response to Ang 1-7 was observed. The influence of renal sympathetic nerve activity (RSNA) on renal hemodynamics is well-established. Stimulation of these nerves typically results in reduced RBF, a response that can be abolished by angiotensin-converting enzyme inhibitors (ACE-Is) (Handa and Johns 1985). However, the impact of renal denervation on renal hemodynamic responses remains a topic of debate. Some evidence suggests that basal RSNA does not significantly affect renal hemodynamics, and renal denervation does not alter RBF or RVR (Luippold et al., 2004; Sadowski et al., 1979). This implies that under normal physiological conditions, the tonic influence of the renal nerves on renal vasculature may be minimal, with the role of these nerves in water and salt retention being more pronounced than their effect on renal vascular dynamics and RBF (Kazi 2010). In our study, we observed no changes in basal renal parameters, including RPP, RBF, and RVR, following acute denervation in either the saline- or losartan-treated groups before Ang 1-7 infusion in both sexes. This finding is consistent with previous studies (Abdulla et al., 2008a; Abdulla et al., 2008b; Dibona and Sawin 2004; Kazi 2010; Rudd et al., 1986; Takabatake et al., 1990). Similarly, in normal animal models, where basal RSNA has been shown to have a sub-vasoconstrictor effect, neither basal RBF nor dynamic RBF auto-regulation were affected by the elimination of basal RSNA through renal sympathectomy (ABILDGAARD et al., 1986; Abu-Amarah et al., 1998; Bello-Reuss et al., 1975; DiBona and Rios 1980; Dibona and Sawin 2004; Just et al., 1998; Osborn et al., 1981). However, in cases where basal RSNA levels exert a renal vasoconstrictor effect, renal denervation has been found to disrupt RBF dynamic auto-regulation, suggesting that the degree of basal RSNA-mediated vasoconstriction contributes to the maintenance of normal RBF dynamic auto-regulation (Grady and Bullivant 1992; MALPAS and EVANS 1998; Malpas et al., 1998).

In the present study, no changes in RBF and RVR in response to Ang1-7 were observed in the saline-treated groups of both sexes. This finding aligns with previous studies suggesting that under physiological conditions, Ang1-7 does not significantly impact kidney hemodynamics (Gorelik et al., 1998; Stegbauer et al., 2004; van der Wouden et al., 2006). Further evaluation of Ang1-7 efficacy on rat renal blood vessels in in vitro and in vivo experimental settings has shown that while Ang1-7 itself does not directly affect basal renal vasomotor tone, it does inhibit AngII-induced contraction of renal arteries in vitro (van der Wouden et al., 2006). However, it does not exert a primary role in regulating in vivo RBF, especially when it is already reduced by AngII in normotensive rats (Handa et al., 1996; van der Wouden et al., 2006). Nonetheless, it has been demonstrated that Ang1-7 can dilate afferent arteries by stimulating nitric oxide (NO) (Ren et al., 2002). These findings suggest that it is hard to induce renal hemodynamic effects of Ang1-7 in vivo. Regarding RBF is controlled by many vascular regulating factors, so the vasorelaxation effects of Ang1-7 may be covered in vivo (Navar et al., 1996). Inconsistent results of Ang1-7 effects observed in vitro versus in vivo settings can be attributed to several factors, including the presence of nerve fibers, circulating humoral substances, and the regulation of blood pressure (Liu and Barajas 1993). Additionally, differences among species, local and systemic concentrations of Ang1-7, water and sodium status, and the level of activity of the RAS can contribute to the contradictory renal responses to Ang1-7 (Pinheiro and Simões e Silva 2012). The systemic vasorelaxation effects of Ang1-7 may not be evident if the baroreceptor reflex is intact or if the endogenous RAS is not activated (Moon 2011). Moreover, the activity of the endogenous RAS can influence the renal response to exogenous Ang1-7 (Barry et al., 2021; O'Neill et al., 2013; O'Neill et al., 2017).

However, after infusion of losartan, there was a considerable increase in RBF response to Ang1-7 infusion in male rats, but the severity of this response was reduced by renal denervation. Also, as expected, the RVR in this group decreased significantly. Consistent with our results, it has been shown that concurrent treatment with Ang1-7 and AT1R inhibitors leads to an increase in RBF (Heller et al., 2000). Additionally, studies have demonstrated that the blunted vasorelaxation induced by Ang1-7 can be restored by acute or chronic blockade of the AT1R receptor with losartan in hypertensive animals (Santos 2014). Studies have shown that serum levels of Ang1-7, as well as Ang IV, increased by treatment with AT1R antagonists. Besides, Ang IV contributes to

the vasodilatory effects of AT1R antagonists through the AT4 receptor (Hilgers and Mann 2002). Also, AT1R antagonists over-stimulate the AT2R (Hilgers and Mann 2002), and activation of AT2R can also increase BK levels, e.g., in vessels (Tsutsumi et al., 1999) or kidneys (Siragy et al., 1999). According to the findings, the vasodilatory effects of Ang1-7 were due to BK and the mechanisms related to releasing NO (Almeida et al., 2000; Ueda et al., 2001). Other data confirm the synergistic effect of losartan and Ang 1-7 on improving renal function in ischemia-reperfusion injury (Safari et al., 2019) and cardiovascular protection (Collister and Hendel 2003) through NO-related AT2- and Mas-receptors-dependent mechanisms (Iyer et al., 1998; Ren et al., 2002; Souza et al., 2013; Stegbauer et al., 2003). Of course, there are conflicting results with our findings. These investigations established that vasorelaxant effects of Ang1-7 were weakened by AT1R antagonist losartan (Gorelik et al., 1998; Neves et al., 2003; Yousif et al., 2017). Also, AT1R blockade did not modify the Ang1-7-activity on BK (Fernandes et al., 2001) and indicated that AT1R does not exert a prominent role in the renal reaction to exogenous Ang1-7 (Vallon et al., 1998). However, in the existing study, RDN weakened the synergistic reactions of losartan and Ang1-7 on RBF. RDN rats indicated reductions in renal tissue concentrations of BK and AngIV, along with Ang II concentration (Bohlender et al., 2017). This may be one of the possible reasons for the observed results in this study. On the other, RBF and RVR response to Ang 1-7 infusion did not change in any of the saline- and losartan-treated female groups. Additionally, there were no significant differences between innervated and denervated groups. Mas receptor (MasR) expression and the ratio of AT2R to AT1R (AT2R/AT1R) are higher in women than in men (Sampson et al., 2012). Studies have shown that the renal vascular response to Ang1-7 is also related to gender and sex hormones. Although the MasR is known to be the specific receptor for Ang1-7, injection of the MasR antagonist (A779) may reduce the RBF response to Ang1-7 in females, while no such observation was made in males (Nematbakhsh and Safari 2014).When the MasR was blocked, the RBF response to Ang1-7 was reduced in ovariectomized estradiol-treated female rats compared with non-estradiol-treated rats, by an unknown mechanism (Saberi et al., 2016). Different results regarding the effect of Ang1-7 on RBF (Bürgelová et al., 2002; Handa 2000; Potthoff et al., 2014) and the sex-specific response of RBF to Ang1-7 have been reported (Nematbakhsh and Mansouri 2018; Nematbakhsh and Safari 2014). Furthermore, inhibiting the Ang II receptors (AT1R and AT2R) and the Ang1-7 receptor (MasR) increased the RBF response to Ang1-7 injection in males but not in females (Nematbakhsh and Mansouri 2018). These findings support an interaction between MasR and Ang II receptors in the renal circulation. It is worth mentioning that blocking Mas receptors had an effect similar to blocking estradiol in preventing the effects of estradiol. In fact, it seems that the MasR is necessary for the activation of NO production and estradiol-mediated vasodilation (Sobrino et al., 2017).

It has been reported that the vasodilatory effects of Ang 1-7 vary at different estrous phases; it does not significantly affect diestrus but has a moderate vasodilatory impact on proestrus. Therefore, estrogen supplements can significantly affect the dilative effect of Ang1-7 (Neves et al., 2004). Female sex hormones probably regulate AT2R (Armando 2002). Thus, the RAS status, besides the status of reproductive hormones, can determine the induction of Ang1-7 vasodilatory response in females (Saberi et al., 2016).

In this study, infusion of Ang 1-7 induced a notable increase in MAP in male and female rats treated with saline. However, blocking AT1R eliminated this pressor effect, which is consistent with other results (Kuczeriszka et al., 2018; Saberi et al., 2016). This suggests that Ang1-7 exerts weak vasoconstrictor effects mediated by the AT1R receptor (Handa et al., 1996; Kuczeriszka et al., 2018). Conversely, insignificant effects of Ang1-7 on MAP were also observed (Braga et al., 2002; Crackower et al., 2002; Vallon et al., 1998). In this study, female rats showed a decrease in pressure after RDN, while in male rats, there was no change in pressure. RDN rearranges or restores the baroreflex control of RSNA (Khan et al., 2014; Messerli and Bangalore 2014). The baroreflex sensitivity (BRS) reflects the rate of baroreflex action, vascular resistance, and vascular tone. Ang1-7 infusion changed the BRS in renal denervation in a sex-dependent manner (Azadbakht and Nematbakhsh 2017).

Conclusion

This study aimed to investigate the role of renal sympathetic nerves and Ang1-7 in regulating renal hemodynamics, particularly in the presence of AT1 receptor

blockade. To achieve this, RBF was assessed in both innervated and denervated rats infused with losartan and without losartan, across both sexes. Interestingly, losartan significantly enhanced the renal blood flow response to Ang1-7 in male rats. However, renal denervation attenuated this response, and no significant reaction to Ang1-7 was observed in any of the female groups studied. Notably, female rats, potentially due to decreased estrogen levels, exhibited a diminished Ang1-7 vasodilator response during diestrus. Additionally, Ang1-7 was found to exert weak vasoconstrictor effects mediated by the AT1R. Overall, RDN and gender emerged as factors influencing the response to Ang1-7 infusion.

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Conflict of interest

The authors declare no conflicts of interest.

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