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Original Article

Role of Mas receptor in renal blood flow response to angiotensin-(1-7) in ovariectomized estradiol treated rats

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Abstract

The angiotensin 1-7 (Ang 1-7), is abundantly produced in kidneys and antagonizes the function of angiotensin II through Mas receptor (MasR) or other unknown mechanisms. In the current study, the role of MasR and steroid hormone estrogen on renal blood flow response to Ang 1-7 administration was investigated in ovariectomized (OV) female rats. OV female Wistar-rats received estradiol (500 µg/kg/week) or vehicle for two weeks. In the day of the experiment, the animals were anesthetized, cannulated, and the responses including mean arterial pressure, renal blood flow (RBF), and renal vascular resistance at the constant level of renal perfusion pressure to graded infusion of Ang 1-7 at 0, 100 and 300 ng/kg/min were determined in OV and OV estradiol-treated (OVE) rats, treated with vehicle or MasR antagonist; A779. RBF response to Ang 1-7 infusion increased dose-dependently in vehicle (P_{dose}<0.001) and A779-treated (P_{dose}<0.01) animals. However, when MasR was blocked, the RBF response to Ang 1-7 significantly increased in OV animals compared with OVE rats (P<0.05). When estradiol was limited by ovariectomy, A779 increased RBF response to Ang 1-7 administration, while this response was attenuated in OVE animals.

Keywords: Estradiol; Renal blood flow; Angiotensin 1-7; MasR

INTRODUCTION

Angiotensin-(1-7) (Ang1-7), the active heptapeptide of renin-angiotensin system (RAS), is known to play an important role in kidney function beside angiotensin II (Ang II) (1). The known receptor of Ang1-7 called Mas receptor (MasR), is usually considered as AngII type 1 receptor antagonist (2-4). Ang1-7 has vasodilatory, antihypertensive, and antiprolifrative activitities on the contrary of Ang II, apart from its tubular activity (5). Moreover, it decreases the presser response to Ang II in renal afferent arterioles of rabbits (6). Sampaio and cowokwers reported that infusion of Ang1-7 in low concentration can increase renal blood flow (RBF) without change in blood pressure in rats (7). Nematbakhsh and Safari showed that the RBF response to Ang 1-7 administration was increased both in male and female animals, but MasR blockade attenuated this reopnsein female rats (8). MasRis also involved in renal function and kidney hemodynamic in both genders (9).

Gender and estrogen are two major determinants of RAS function (10). It is well known that vasodepressor arm of RAS is more potent in the females while vasopressor arm is more potent in males (11,12). In addition, the activity of angiotensin converting enzyme 2 (ACE2); the major enzyme in synthesis of Ang1-7 in kidney is also gender-related. It seems that the interact effects between Ang1-7 or Ang II and female sex hormone is a complex pathway and the exact mechanisms need to be defined. It is seen that plasma Ang1–7 level elevates in normal pregnancy (13), and the level of Ang II in plasma is higher in oral contraceptive users with a modest alteration in hemodynamic (14). Unexpectedly, different responses to Ang II between males and females when both Ang II type 2 receptor (AT2R) and MasR were

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blocked was observed (15), which reveals the interaction between the receptors. It is documented that AT2R is upregulated by estrogen (16). However, the role of female sex hormone on regulation of Ang1-7 receptor; MasR, is not well determined. Based on this rational, we hypothesized that estrogen may alter RBF response to Ang1-7 infusion via its effect on MasR. To test this hypothesis, MasR antagonist (A779) was administered in ovariectomized estradiol-treated (OVE) ratsand the RBF response to Ang1-7 was compared with the control group.

MATERIALS AND METHODS

Animals

Twenty six ovariectomized female Wistar rats weighing 200 ± 20 g were housed at room temperature of 23-25 °C with a 12 h light/dark cycle. The rats were fed with rat chow and had acces to tap water *ad libitum*. All animal experiments were approved by the Ethics Committee of Isfahan University of Medical Science and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals.

Surgical procedure for the ovariectomy

Female wistar rats were anaesthetized with 0.06 g/kg of 10% ketamine and 2% xylazine solution (5:2) (Alfasan Co. Woerden, Holland). The suprapubic region was scrubbed with betadine, and a small incision was made. The ureter near the ovaries was tightly occluded, ovaries were removed, incision was sutured, and the animals were housed for one week to recover.

Experimental surgery

The rats were anaesthetized with urethane (1.7 g/kg, Merck, Germany) and trachea was cannulated to facilitate air ventilation. The carotid and femoral arteries and jugular vein were cannulated by polyethylene microtube (Microtube Extrusions Pty Ltd, Australia). Jugular vein was used for drug infusion and arterial catheters were connected to pressure transducers and a bridge amplifier (Scientific Concepts, Vic., Melbourne, Australia) to record mean arterial pressure (MAP) and renal perfusion pressure (RPP) by the carotid and femoral arteries, respectively. An adjustable clamp was placed around the abdominal aorta above the renal artery to control RPP during Ang1-7 administration. The left kidney was exposed and fixed in a special kidney cup. Renal artery was carefully separated from the renal vein. The transit-time ultrasound flow probe interfaced with a compatible flowmeter (T108; Transonic Systems) was placed around the renal artery to measure RBF directly. MAP, RPP, and RBF were continuously measured throughout the experiment and the data were recorded with 2 s intervals via a data acquisition system. The animals with RPP less than 90 mmHg were excluded from the study. Body temperature monitored by a control unit (Model HB101/2; AgnTho's AB, Lidingo, Sweden) and maintained in the normal range (36.5-37.5 °C) throughout the experiment. We allowed a 30 to 60 min period for equilibration before experimental interventions. Renal vascular resistance (RVR) was calculated by dividing RPP to RBF. The bladder was canulated by making an incision on the suprapubic region and placement of a cannula to drain urine during experiment.

Experimental protocol

The ovariectomised animals were divided into two groups. Group 1 (OVE, n = 12) received intramuscular injections of 500 µg/kg/week estradiol valerate (Es, Abureihan, Tehran, Iran) dissolved in sesame oil for two weeks. Group 2, ovariectomized female rats (OV, n = 13), received intramuscular injections of sesame oil as the vehicle for two weeks.

After the equilibrium period, the animals in each group (either OVE or OV) were further divided into two subgroups. Therefore, four groups of animals were used in this study which are categorized as follows:

Group 1A, OVE rats (n = 6) treated withA779; Group 1B, OVE rats (n = 6) received saline as the vehicle;Group 2A, OV rats (n = 6) were treated with A779; and Group 2B, OV rats (n = 7) received saline as the vehicle.

A779 (Bachem Bioscience Inc., King of Prussia, PA, USA),was dissolved in 0.9% w/v saline and administered intravenously by a microsyringe pump (New Era Pump System Inc. Farmingdale, NY, USA) as bolus doses of 50

μg/kg followed by continuous infusions at 50 μg/kg/h. MAP, RPP, and RBF were recorded for 30 min. The last 3-5 min period of measurement was considered as the "effect of antagonist time" and also as the control for Ang1-7 infusion time.

Response to Ang1-7 infusion

Ang1-7 was administered intravenously by a microsyringe pump at two different continuous doses of 100 and 300 ng/kg/min after antagonist/saline infusion. Each dose was infused for 15 min; and MAP, RPP, and RBF were recorded during Ang1-7 infusion and the last 3-5 min of each dose measured as "response to Ang1-7 infusion". During Ang1-7 infusion, RPP was sustained at pre-Ang1-7 infusion levels via an adjustable aortic clamp. At the end of the experiment, the rats were humanely killed by anesthetic overdose, and the left kidneys were removed and weighed immediately.

Statistical analysis

Data was analyzed by the SPSS software, version 16, and expressed as the mean ± SEM. The animals treated with A779 and vehicle were compared with regrds to the basal data for MAP, RPP, RBF or kidney weight for each group by unpaired Student's t-test. The responses to Ang1-7 are reported as percentage (%) of change from the baseline values, and compared via repeated measures ANOVA for the different groups. *P value* ≤0.05 was considered significant.

RESULTS

Baseline measurement

The two groups of OVE and OV either treated with A779 or vehicle were not significantly different in terms of MAP, RPP, RBF, and kidney weight in the equilibrium stage as indicated by baseline measurements (Fig. 1).

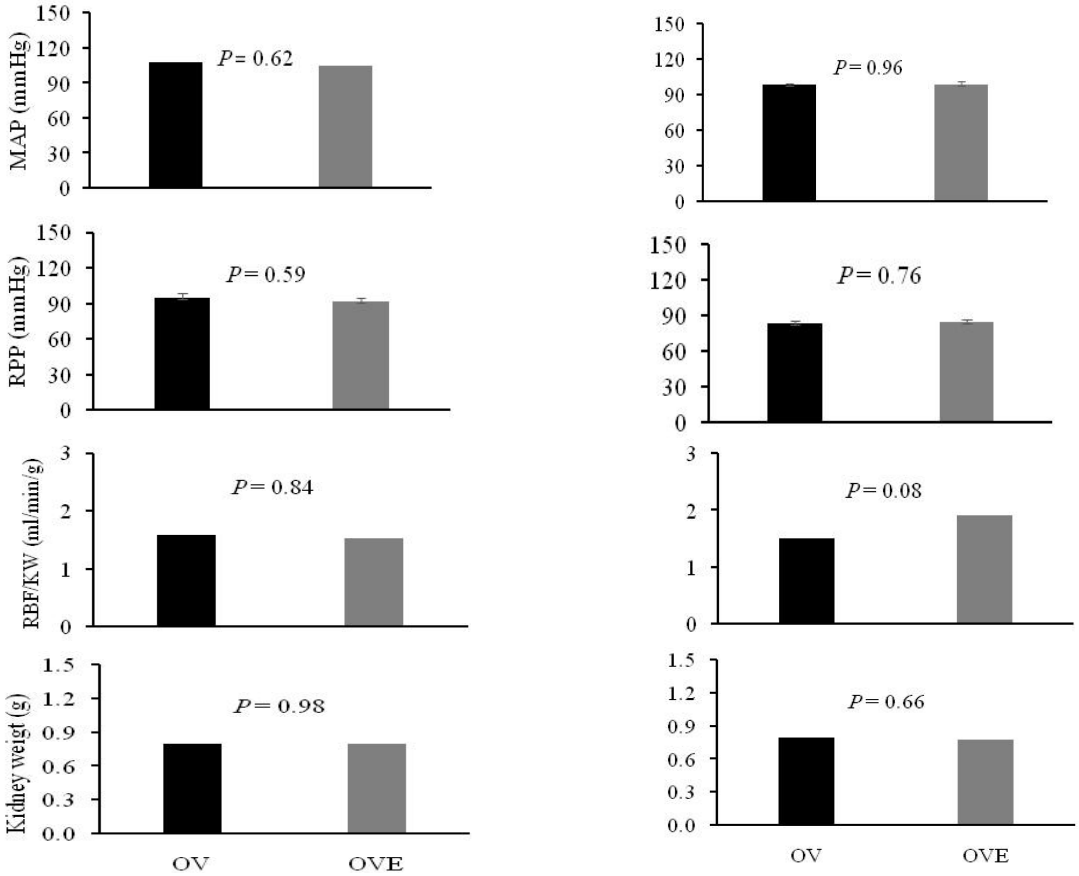

Fig. 1. Hemodynamic parameters in ovariectomized (OV) and ovariectomized estradiol treated (OVE) rats in vehicle (the left panels) and A779 (the right panels) groups as the basal data. Data are presented as mean ± SEM. MAP; mean arterial pressure, RPP; renal perfusion pressure, RVR; renal vascular resistance, RBF; renal blood flowpergram kidney weight.

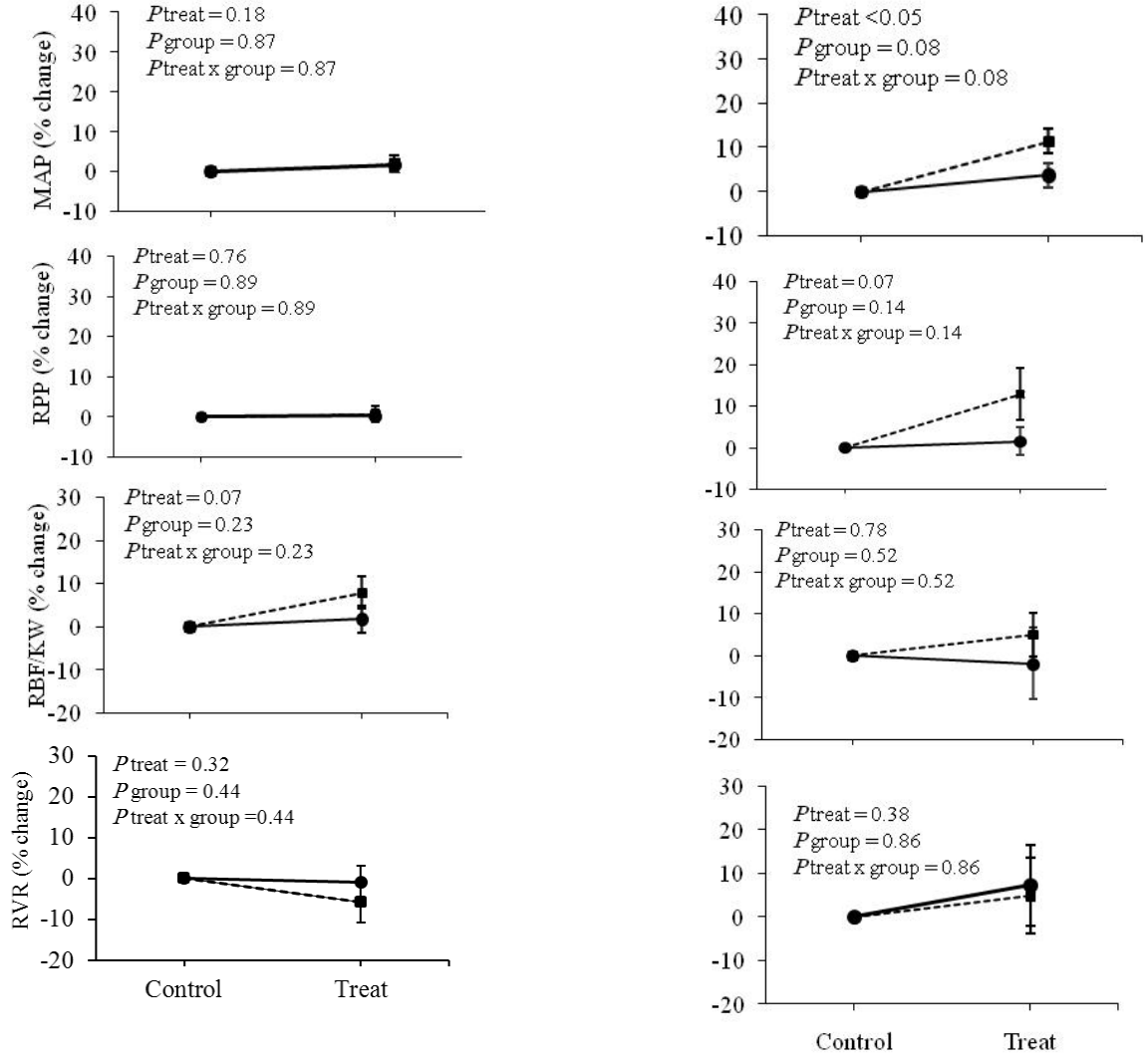

Fig. 2. Hemodynamic parameters in ovariectomized (OV, —●—) and ovariectomized estradiol treated (OVE, – ■ –) rats before and after administration of vehicle (left panel) or A779 (right panel) as effect of antagonist (Treat). Data are presented as mean ± SEM of percentage changes from the baseline. MAP; mean arterial pressure, RPP; renal perfusion pressure, RVR; renal vascular resistance, RBF; renal blood flow per gram kidney weight. The *P* values were derived from repeated measure ANOVA.

Effect of antagonist

The results indicated that vehicle (saline) infusion had no effect on MAP, RPP, RBF, and RVR in both OV and OVE groups. However, A779 infusion significantly increased percentage change of MAP in Es-treated rats ($P<0.05$). RBF and RVR altered significantly while no differences were detected between the groups (Fig. 2). It could be summarized that infusion of saline as well as A779 did not cause statistically significant alterations in RBF or RVR, and the OV or OVE groups were not significantly different in this respect (Fig. 2).

Responses to Ang1-7 infusion

Ang1-7 infusion significantly increased the percent changes of MAP in vehicle-treated ($P_{dose}<0.01$) and in A779-treared groups ($P_{dose}<0.001$) but there were no statistically significant difference between OV and OVE groups (Fig. 3). As explained before, RPP remained at the basal level by clamp placed around the aorta above the renal arteries. Therefore, percent change in RPP was almost constant during Ang1-7 infusion with no significant differences between saline- and A779-treated animals in both OV and OVE groups (Fig. 3). Ang 1-7 infusion increased

percent changes in RBF dose-dependently in vehicle- (P_{dose}<0.0001) and in A779-treated animals (P_{dose}<0.001). However, this increase was not statistically significant in vehicle subgroups, and surprisingly, percent change of RBF was increased by Ang1-7 in the OV group, which was statisticaly different from that in the OVE group (P = 0.028). For instance, infusion of 300 ng/kg/min of Ang1-7, when MasR was blocked, increased the percent change of RBF by 7.86 ± 2.7 in the OVE group while RBF increased by 22.4 ± 6.5 in the OV group. Ang1-7 infusion decreased percent changes of RVR dose-dependently in vehicle- (P_{dose}<0.01) and A779- treated animals (P_{dose} = 0.10). However, no significant differences were observed between the groups.

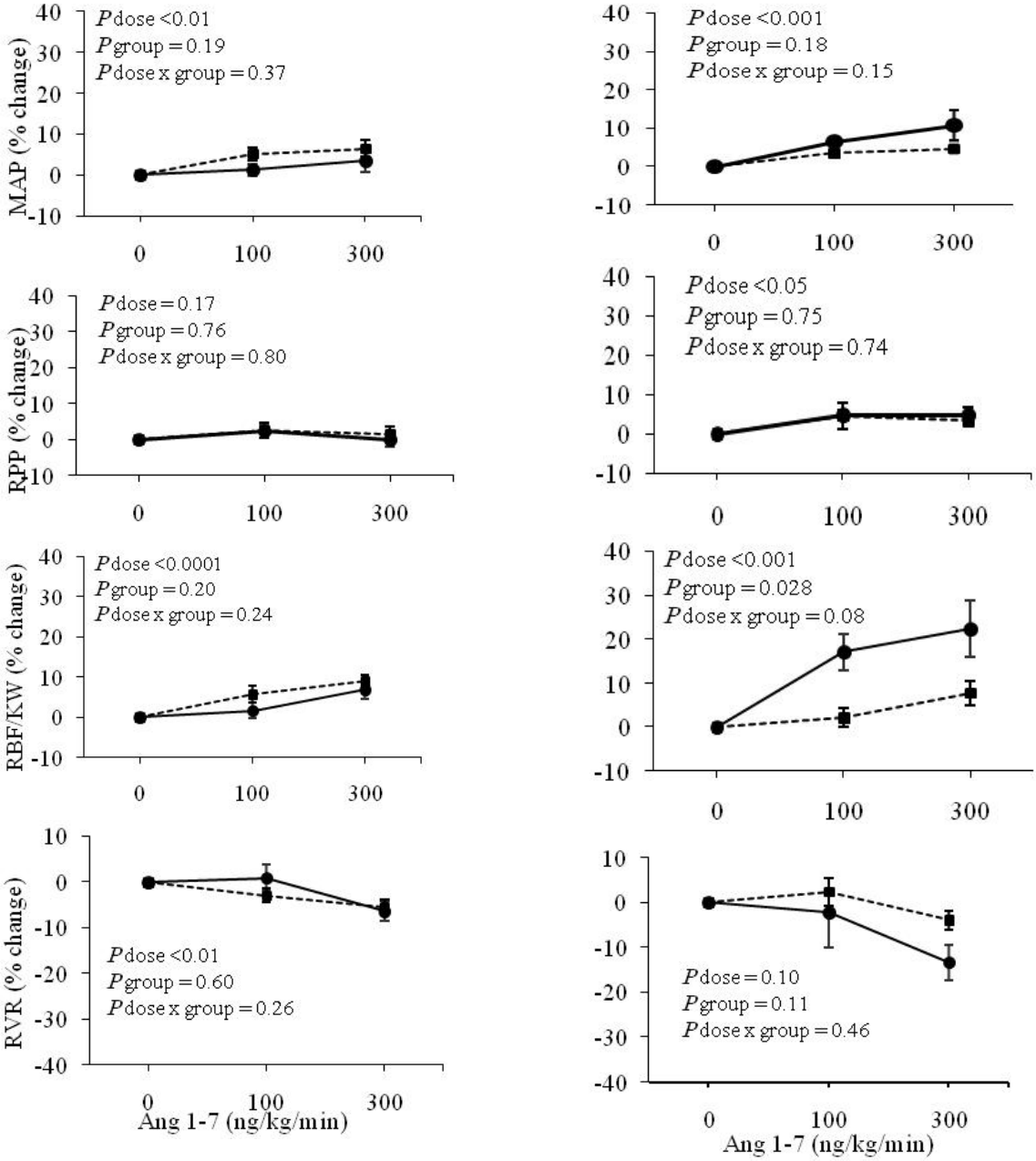

Fig. 3. Effects of vehicle (left panels) or A779 (right panel) on responses to angiotensin 1-7 infusion in ovariectomized (OV,—●—) and ovariectomized estradiol treated (OVE, – ■ –) rats. Data are presented as mean ± SEM of percentage changes from MAP; antagonistinmean arterial pressure, RPP; renal perfusion pressure, RVR; renal vascular resistance, RBF; renal blood flow per gram kidney weight. *P* values were derived from repeated measure ANOVA.

DISCUSSION

In this study, we examined the renal hemodynamic response to Ang 1-7 infusion in the presence and absence of MasR antagonist (A779) in ovariectomized rats treated with estradiol and compared with control animals. Our major findings indicated that in the presence of MasR, RBF response to Ang 1-7 infusion increased in a dose dependent manner in both estradiol or non-estradiol treated rats. However, this response increased significantly in non-estradiol treated compared to the estradiol-treated rats when MasR was blocked. These findings were unexpected , since MasR was blocked and the circulatory estrogen level was in the minimum possible level in non-estradiol treated animals. To interprete this finding, first we need to consider the effect of Ang 1-7 on RBF under normal condition, and then include estradiol and MasR effects.

Sampaio and colleagues evaluated the regional and systemic blood flow and vascular resistance changes in response to intravenous infusion of Ang 1-7 (7). They reported that low dose of Ang 1-7 (110 fmol/min/10min) increased blood flow in kidney, brain, and mesentery while vascular resistance alteration in these organs inversely correlated with blood flow changes although high dose Ang 1-7 infusion (11 pmol/min/10 min) showed an increase in vascular resistance and a decrease in renal, mesenteric, and cutaneous blood flow (7). In addition, A779 significantly reduced blood flow in the mentioned organs, and augmented vascular resistance while the effect of low dose of Ang 1-7 was abolished completely (7). The lower total peripheral resistance was found in chronic exposure to Ang 1-7 while no alteration was seen in MAP (17). On the other hand, different result indicated that Ang 1-7 has no effect on renal blood flow (18). However, it seems that Ang 1-7, which is abundantly produced in the kidneys (19), has an important role in modulating renal blood flow.

Estradiol may also alter the Ang 1-7 level. In proestrus and oestrus phases, the plasma level of Ang 1-7 is higher than that in the metoestrus and dioestrus phases in the ovary of rat. This finding reveals that Ang 1-7 is regulated by ovarian hormones (20). Liu and coworkers investigated the effect of gender on ACE2 and reported that renal ACE2 activity in male is greater than that in the female kidneys. Without considering the sex chromosome complement, estradiol therapy decrease 60% of ACE2 activity in gonadectomized animals (21). Ji and colleagues reported that ovariectomy decreased ACE2 activity as well as protein and mRNA expression in kidney cortex while treatment with estradiol reversed this phenomenon (22). In the presence of estradiol, treatment with chronic low dose of Ang II, ACE2 activity and mRNA expression increased, but ovariectomy abolished these effects such that estradiol shifted RAS activity toward vasodilatory response (23). However, some findings do not support the existence of greater vasodilatory component (AT2R and ACE2) in females (24). On the other hand, it is reported that ovariectomy had no effect on MasR expression (25). In addition to increasing the endothelial RAS proteins, estradiol contribute to nitric oxide (NO) production and this effect is mediated by MasR (26). The information available on the effect of estradiol on RAS verify our hypothesis that estradiol affects renal hemodynamics. According to our results Ang 1-7 increased RBF by decreasing of RVR, and the role of estradiol was dominant because RBF response was lower in OVE+A779-treated rats compared to OV+A779-treated groups. Acually, when MasR is blocked, estradiol attenuates the RBF response to Ang1-7.

Several researches have explained the role of this hormone on blood flow through alteration of vascular resistance (27,28). Postmenopausal women that received conjugated equine estrogen had a significant lower RBF than the baseline (27). In addition, in postmenopausal women,the effect of acute 17β-estradiol on peripheral blood flow (PBF) and resistance was assessed, and an increase in PBF and a decrease in peripheral resistance was observed (28). Prolonged 17β-estradiol infusion in ovariectomized non-pregnant ewes increased blood flow in reproductive and non-reproductive tissues subsequent to cardiac output elevation and systemic vascular

resistance reduction (29). In non-pregnant sheep, relaxation response to bradykinin and superoxide dismutase in the uterine arteries is seen, which is caused by enhancement of NO release and NO synthase activity by estrogen. This finding was not observed in the renal arteries (30). To summarize the related literatures in this respect, estradiol may increase the level of Ang 1-7 and increase renal blood flow via decreasing vascular resistance.

Contribution of estrogen in vascular response to Ang 1-7 in different phases of estrus cycle was assessed in isolated mesenteric artery by Neves and colleagues. They observed that Ang 1-7 dilates the endothelin-1 preconstricted mesenteric artery in a dose-dependent manner in ovariectomized estradiol-treated rats and a modest vasodilation was seen in virgin rats at proestrus, but Ang 1-7 had no effect on arterial diameter in ovarectomized placebo-treated and virgin rats at diestrus (31). Dilatory response of estradiol-treated ovariectomized rats was abolished by pretreatment with D-Ala-Ang 1-7, whereas this agent had no effect on ovariectomized non-estradiol-treated rats at diestrus and proestrus phases (31). Ang 1-7 also dilated mesenteric vessels in pregnant animals but had no effect on isolated mesenteric vessels from virgin rats at diestrus phase, and pretreatment with MasR antagonist prevented dilatory response of Ang 1-7 also (32). Ang 1-7 itself is a vasodilator agent that inserts its effects through MasR. All these studies investigated the above-mentioned parameters separately while in our study, we investigated the RBF response to Ang 1-7 under both conditions of absence and presence of estradiol and MasR. When MasR was present, RBF response to graded Ang 1-7 infusion increased and RVR decreased while the response was greater in estradiol-treated rats as expected. Surprisingly, when MasR was blocked by A779, the RBF response was significantly higher in non-estradiol-treated rats. At this point, we do not have an exact explanation for this unexpected result. Possibly when MasR is blocked and estradiol is at the minimum possible level in the circulation, Ang 1-7 administration insert its effect via different pathways, based on this concept that Ang 1-7 actions are mediated through NO, prostaglandins (33), and bradykinin (34) via other unknown receptors. However, further studies are required to reveal the exact underlying mechanism and to explain our observations.

CONCLUSION

In OV group that estradiol level is limited by ovariectomy, MasR blockade A779 increased RBF response to Ang 1-7 administration, while this response was attenuated in OVE group.

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