# Angiotensin 1-7 Administration Increases Renal Blood Flow in the Absence of Bradykinin B2 Receptor in Ovariectomized Estradiol Treated Rats: The Role of Mas Receptor

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**Abstract**- Renin angiotensin (RAS), kallikrein kinin (KKS), and sex hormonal systems demonstrate a complex contribution in kidney circulation. This study was designed to investigate the role of angiotensin 1-7 (Ang 1-7) receptor (MasR) and of bradykinin B2 receptor (B2R) in renal blood flow (RBF) response to Ang 1-7 infusion in ovariectomized estradiol treated rats. The ovariectomized rats received intramuscular vehicle (group 1, OV) or estradiol valerate (500 µg/Kg/week) (group 2, OVE) for two weeks. Then each group was divided into two subgroups subjected to receive B2R antagonist (HOE-140, subgroup A), or MasR antagonist (A779) plus HOE-140 (subgroup B). RBF and renal vascular resistance (RVR) responses to graded Ang 1-7 infusion were determined. In condition of B2R alone blocking, RBF response to Ang 1-7 in OVE group was significantly greater than that of OV group ($P$=0.05), however this response difference was failed by co-blockades of MasR and B2R. Estradiol could promote RBF response to graded Ang 1-7 infusion in the absence of B2R alone, however when both receptors (MasR and B2R) were blocked the role of estradiol was limited.



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## Introduction

Renin angiotensin (RAS) and kallikrein-kinin (KKS) systems contain several peptides, enzymes, and receptors. Angiotensin 1-7 (Ang1-7) and its specific Mas receptor (MasR) is one of the components of RAS, while bradykinin (Br) with two specific receptors of B1 and B2 belongs to KKS.

Both RAS and KKS are present in the kidney and play an important role in renal hemodynamic regulation (1,2). Br induces a transient increase in renal blood flow (RBF) (3), and it has a biphasic effect on afferent and a dilatory effect on efferent arteries (4,5). RAS and KKS have multiple cross-talks and the components of these two systems could increase in the same direction or show a counter regulatory effect (6,7), angiotensin converting enzyme (ACE) is an intermediate enzyme in both systems (8). Ang 1-7 potentiates Br effect (9,10), and increases Br $B_2$ receptor ($B_2R$) mRNA and protein expression (11).

Ovariectomy decreased Br mRNA level in kidney and aorta, while estrogen restored it to normal level (12). In pregnancy and estrus cycle in which estrogen level is high, Br increases, so that the uterine and uteroplacental blood flow elevate. On the other hand, RAS is structurally and functionally affected by estrogen, and estrogen increases ACE2 activity and Ang 1-7 production (13-15), We hypothesized that MasR, B2R, and estradiol have a complex contribution to RBF response to Ang 1-7 infusion. To clarify this complexity, the B2R was blocked and the role of MasR on RBF response to Ang 1-7 administration in ovariectomized rats threated with estradiol were evaluated.

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## Materials and Methods

### Animals

The experiments were performed on 26 ovariectomized female Wistar rats (200±20 g), which were housed at the room temperature of 23–25° C with a 12-h light/dark cycle. The experimental procedures were in advance approved by the Isfahan University of Medical Science's Ethics Committee.

### Surgical procedure

### Ovariectomy

The rats were anaesthetized with 0.06 g/kg of 10% ketamine and 2% xylazine solution (5:2). A small incision was made on the suprapubic region. The ovaries were removed and the incision was sutured. The animals were allowed to recover for one week, and then they were divided into two major groups. Group 1 (OV) received intramuscular injection of sesame oil alone as vehicle for period of two weeks. Group 2 (OVE) received intramuscular injection of estradiol valerate (500 µg/Kg/week, Abureihan, Tehran, Iran) dissolved in sesame oil for period of two weeks.

### Experimental surgery

After two weeks of estradiol therapy, the rats were anaesthetized with urethane (1.7 g/kg; Merck, Germany). Trachea was cannulated to facilitate air ventilation. The carotid and femoral arteries were cannulated by polyethylene microtube to record mean arterial pressure (MAP) and renal perfusion pressure (RPP) after connection to pressure transducers and a bridge amplifier (Scientific Concepts, Vic., Melbourne, Australia). The jugular vein was cannulated for drug infusion. In order to control RPP during Ang 1-7 administration, an adjustable clamp was placed around the abdominal aorta above the renal artery. The left kidney was exposed and fixed in a special kidney cup. To measure RBF, the transit-time ultrasound flow probe was placed around the renal artery and interfaced with a compatible flow meter (T108; Transonic Systems). MAP, RPP and RBF were measured continuously throughout the experiment. Body temperature was 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. The bladder was cannulated to drain the urine during the experiment. We allowed 30–60 min for equilibration before interventions. RVR was calculated by RPP/ RBF ratio.

### Experimental protocol

Each group of animals (OV or OVE) was divided into two subgroups of A and B. After the equilibrium period, subgroups A were treated with B2R antagonist; HOE-140 (Tocris Bioscience, Tocris House, IO Center, UK), and subgroups B received MasR antagonist; A779 (Bachem Bioscience Inc., King of Prussia, PA, USA) plus HOE-140. HOE-140 and A779 dissolved in 0.9% w/v saline and administered 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. In summary, the group design and the treatment of each subgroup is shown in table 1.

MAP, RPP, and RBF measurement for the first 30 min of antagonist's administration were considered as time for antagonists effects. However, antagonist's infusions were continued until end of experiment.

**Table 1. Summary of study design**

| Group | Subgroup | n | Treatment |
|---|---|---|---|
| **1 (OV)** | 1A | 6 | vehicle, HOE-140, Ang1-7 |
| | 1B | 6 | vehicle, HOE-140+A779, Ang1-7 |
| **2 (OVE)** | 2A | 6 | estradiol, HOE-140, Ang1-7 |
| | 2B | 8 | estradiol, HOE-140+A779, Ang1-7 |

### Response to Ang1-7 infusion

To roll out the antagonist's effects, 30 min after antagonists infusion, MAP, RPP and RBF were determined. Then, Ang 1-7 was administered intravenously by a microsyringe pump in three doses of 100, 300, and 1000 ng $kg^{-1}$ $min^{-1}$. Each dose was given for 15 min, and MAP, RPP, and RBF were recorded during Ang 1-7 infusion and the last 3-5 min of each dose were considered as "response to Ang 1-7 infusion". During Ang 1-7 infusion, RPP was sustained at pre-Ang 1-7 infusion levels via an adjustable aortic clamp. At the end of the experiment, blood sample was obtained via heart puncture, and the rats were humanely killed by anesthetic overdose, left kidneys was removed and weighed immediately.

### Statistical analysis

The data was analyzed by the SPSS software, version 16, and expressed as mean ± SEM. The unpaired Student's t-test and repeated measures ANOVA were

employed to compare the parameters between the groups or subgroups. $P \leq 0.05$ was considered significant.

## Results

### Baseline measurement

The baseline data (before antagonist administration) indicated that MAP in all subgroups were in normal ranges, however this pressure was significantly higher in subgroup 1A compared to subgroup 2A ($P$=0.037), but RBF and RPP were not significantly different between these two subgroups. In addition, MAP, RPP and RBF were not significantly different between the subgroups of 1B and 2B (Figure 1, right panel).

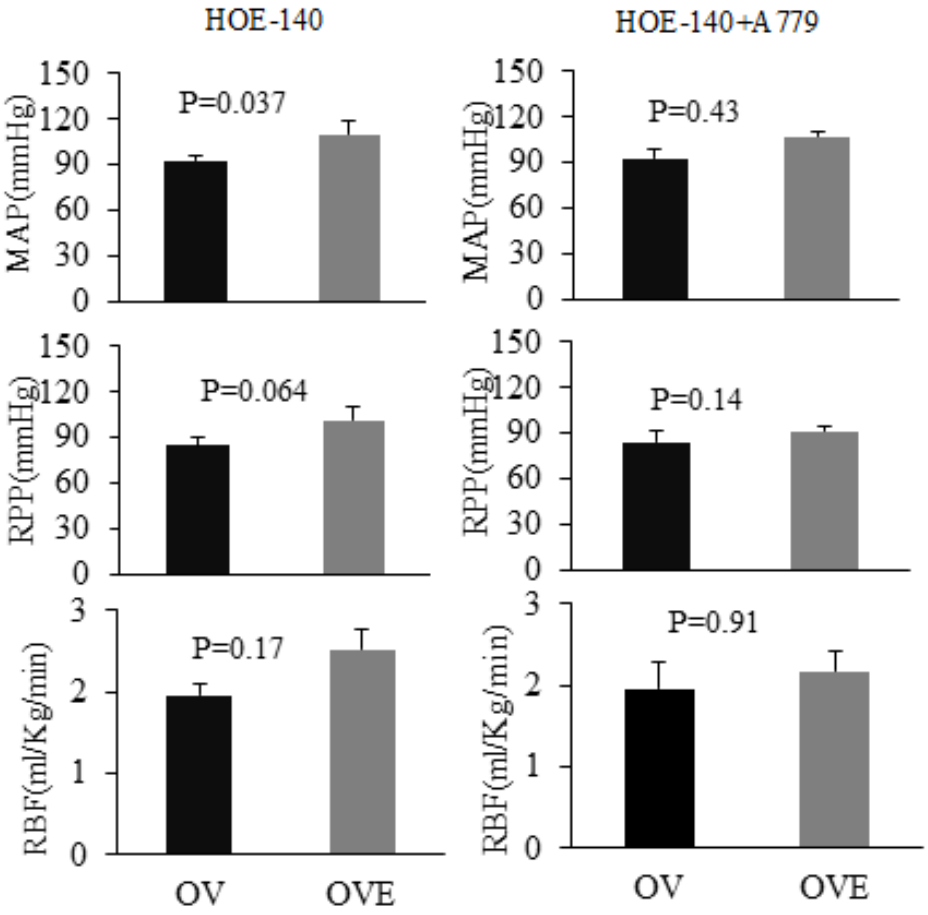


**Figure 1.** Hemodynamic parameters before antagonists administration (baseline data before HOE or A779 administration) in ovariectomized non-estradiol (OV)- or estradiol (OVE)-treated female rats which supposed to treat with HOE-140 (left panel ) and HOE-140+A779 (right panel). MAP: Mean arterial pressure, RPP: Renal perfusion pressure, RBF: Renal blood flow per gram kidney weight. *n* in each group was 6-8. Data are presented as mean ± SEM. The *P* were derived from the Student's t-test

### Effect of antagonists

The effects of antagonists were measured 30 min post antagonists administration. No significant differences were observed between the groups for MAP, RPP or percentage change of RBF (Figure 2).

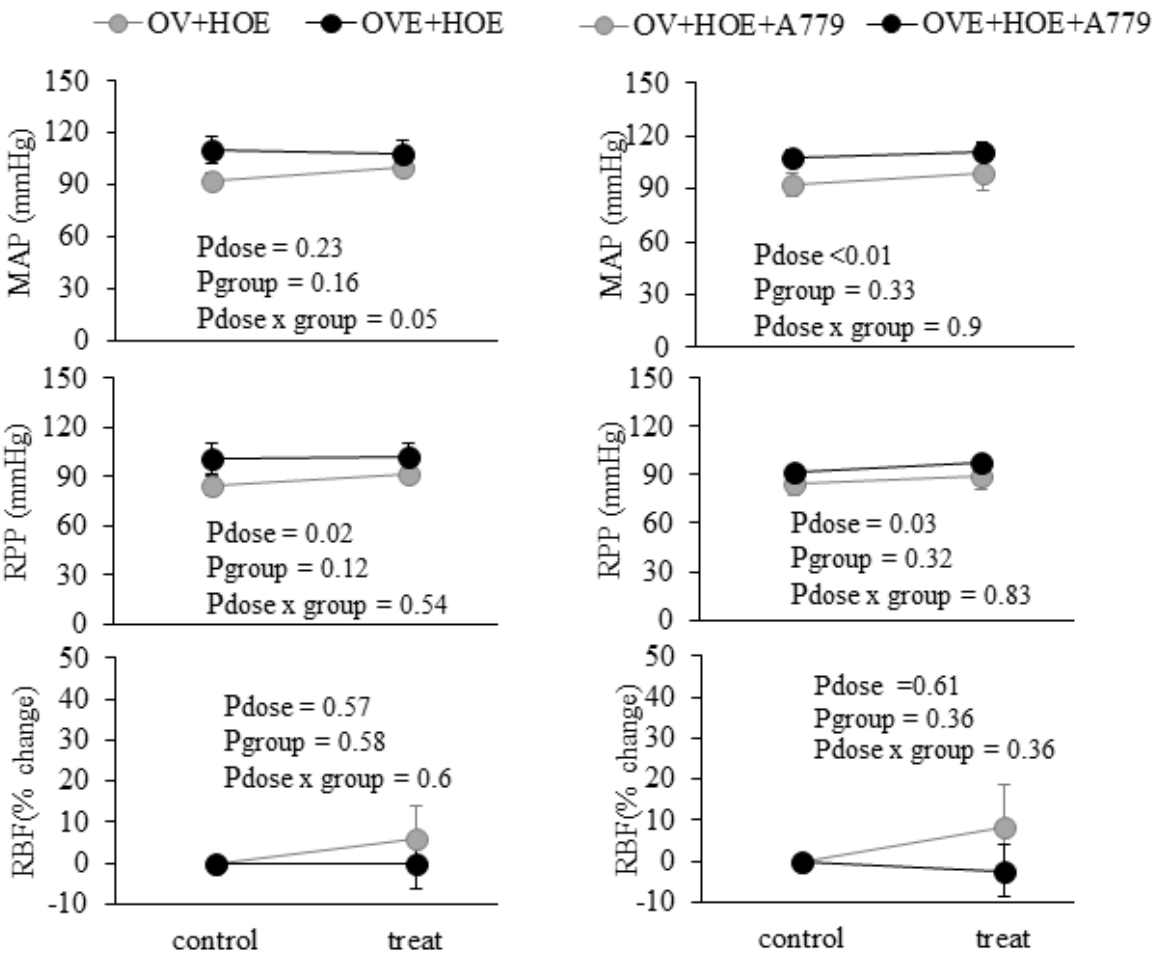


**Figure 2.** Hemodynamic parameters changes after 30 min post HOE-140 or HOE-140+A779 administration (treat) against baseline data (control) as "effect of antagonist" in ovariectomized nonestradiol (OV) or estradiol (OVE) treated female rats. MAP: Mean arterial pressure, RPP: Renal perfusion pressure, RBF: Renal blood flow per gram kidney weight. *n* in each group was 6-8. Data are presented as mean ± SEM. The *P* were derived from repeated measures ANOVA

**Responses to Ang 1-7 infusion**

MAP, RBF and RVR responses to Ang1-7 were compared between each two similar subgroups which were treated with HOE-140 alone (Subgroups 1A and 2A), and with HOE-140+A779 (subgroups 1B and 2B). There was no significant difference in MAP between each two similar subgroups. RPP was maintained at the baseline level by placing an adjustable clamp around the abdominal aorta above the renal artery during Ang 1-7 infusion, which prevented alteration in RPP. Therefore no significant difference in RPP was expected (Figure 3). The percentage changes of RBF (RBF%) response to graded Ang 1-7 infusion increased dose dependently in all the subgroups ($P_{dose}$<0.0001). However, RBF% response to Ang 1-7 in animal treated with HOE-140 and estradiol was significantly greater than that of animal treated with HOE-140 alone ($P_{dose}$=0.05). Such observation was not seen when both MasR and B2R were blocked by A779 and HOE-140 (Figure 3). RVR decreased during Ang 1-7 infusion in a dose dependent manner ($P_{dose}$=0.0001) but no significant difference was seen between the subgroups (Figure 3).

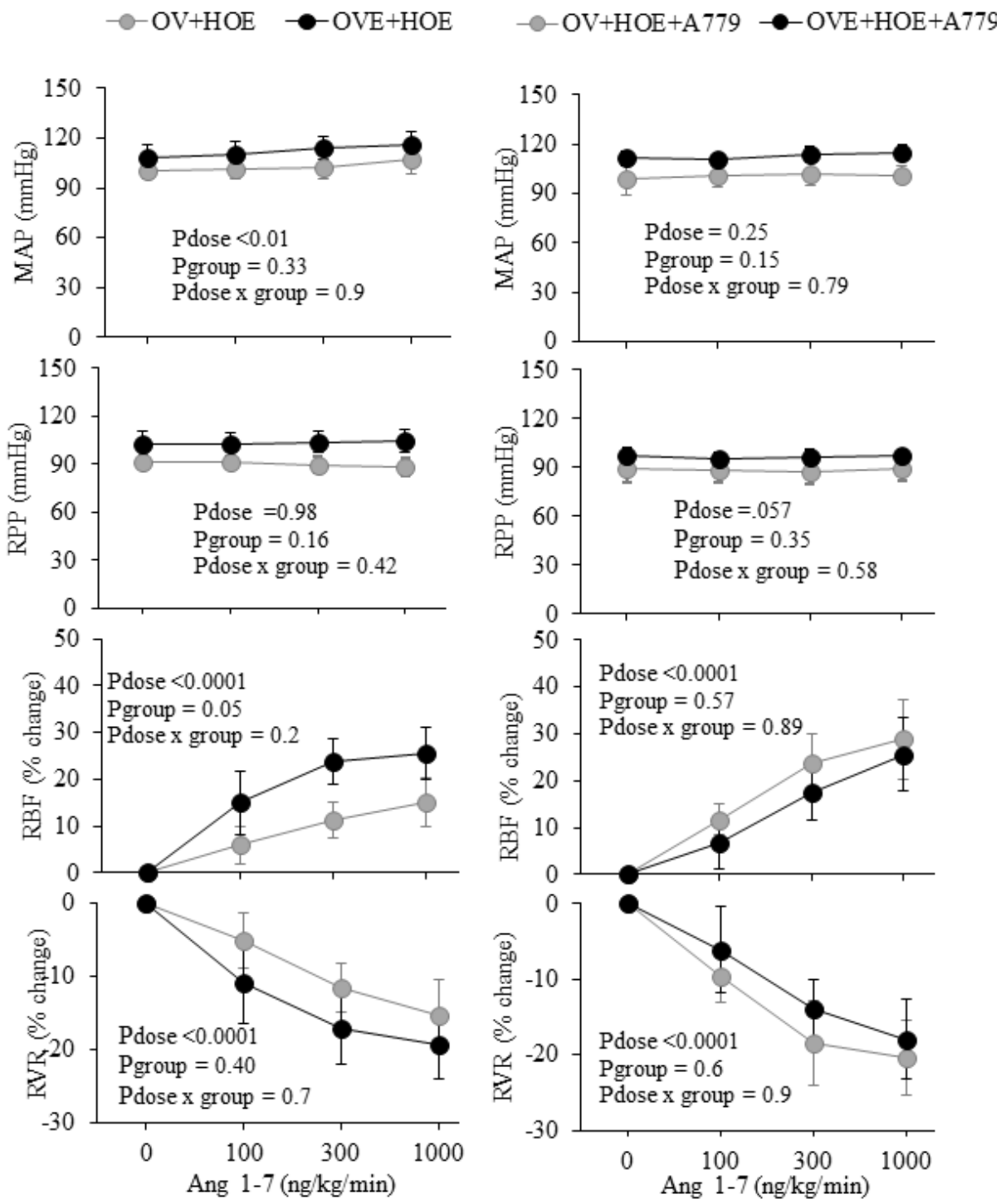


**Figure 3.** Hemodynamic parameters after Ang 1-7 infusion at graded doses in HOE-140 or HOE-140+A779 subgroups in ovariectomized non-estradiol (OV) or estradiol (OVE)-treated rats. MAP: Mean arterial pressure, RPP: Renal perfusion pressure, RBF: Renal blood flow per gram kidney weight. *n* in each group was 6-8. Data are presented as mean±SEM. The *P* were derived from repeated measures ANOVA

## Discussion

We compared the role of MasR in renal vascular response to Ang 1-7 in estradiol and non-estradiol treated ovariectomized rats when B2R was blocked. The major findings indicated that estradiol elevated RBF response to Ang 1-7 when B2R was blocked, and co-blockades of B2R and MasR abolished this response.

MasR is known as specific receptor of Ang 1-7 and the effects of Ang 1-7 exert by this receptor (16). However, it is reported that under normal conditions, RBF response to Ang 1-7 is not essentially depended on MasR (17), and therefore, the role of other parameters such as estrogen, and B2R must be defined. ACE2/Ang1–7/MasR axis is more active in females than males (15,18) while the prominent role of estrogens but not androgens on the vascular tone is emphasized (19,20). Estrogen therapy is reported to improve endothelial function in the forearm arteries in post-menopausal hypertensive women (21). Ang 1-7 and Br interact to regulate vascular tone in the large arteries and in resistance vessels (22,23). Mechanism of this interaction is receptor-mediated as reported that HOE-140 abolishes the potentiation effect of Ang 1-7 on Br while A779 reduces this effect significantly (24). Our results showed when B2R was blocked, RBF response to Ang1-7 was increased dose dependently whether MasR was present or not. However, in the presence of MasR and in the absence of B2R, estradiol is an essential factor that could alter RBF response to Ang 1-7, but when both B2R and MasR were blocked, the role of estrogen was not significant. This finding suggested that possibly, RBF response to Ang 1-7 is neither completely dependent on MasR nor on B2R. However, previous study by Safari et al. showed that co-blocking of Ang II type 2 receptor (AT2R) and MasR not only did not increase RBF response to Ang II but also decreased it (25).

When RAS vasodilator receptors (MasR) and B2R were blocked, the role of estradiol on renal blood flow responses to Ang1-7 was limited. However by B2R alone blocking, the role of estradiol was highlighted. At the present, there is no exact explanation for such observation.

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