Role of Angiotensin Type 2 Receptor on Nitric Oxide Production Response to Angiotensin II Administration in Ovariectomised Rats Treated with Estradiol

Tahereh Safari[1], Mehdi Nematbakhsh[2,3,4]

[1]Department of Physiology, Zahedan University of Medical Sciences, Zahedan, Iran, [2]Water and Electrolytes Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Department of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran, [4]Isfahan [MN] Institute of Basic and Applied Sciences Research, Isfahan, Iran

Correspondence to:
Prof. Mehdi Nematbakhsh,
Water and Electrolytes Research Center/
Department of Physiology,
Isfahan University of Medical Sciences,
Isfahan, Iran.
E-mail: nematbakhsh@med.mui.ac.ir

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Brief Communication

ABSTRACT

Background: Renin-angiotensin system activity is gender related. The vasodilatory response of angiotensin II (AngII) angiotensin type 2 receptor (AT2R) may involve nitric oxide (NO) production. We attempted to find the role of AT2R on NO formation response to AngII administration in ovariectomised rats treated with estradiol (OVE).

Methods: A total of 33 female Wistar rats were divided into 3 groups; intact animals, ovariectomised treated with placebo (OVX) and OVE. At 2 weeks later, all animals were subjected to anesthetize and catheterize and each group was divided into two subgroups that received AT2R antagonist (PD123319) or vehicle. Each animal was subjected to 1 h continuous infusion of AngII (~20 μg/kg/h) and the level of NO metabolite (nitrite) was measured before and after AngII infusion.

Results: At the presence of AT2R, the serum level of nitrite in response to AngII administration in OVE groups increased significantly ($P < 0.05$).

Conclusions: However, this increase was abolished by AT2R antagonist. It seems that AT2R involves nitrite production response to AngII in OVE.

Keywords: Angiotensin receptor, estradiol, renin-angiotensin system, nitrite

INTRODUCTION

Gender differences has been observed in hypertentension, cardiovascular and kidney diseases.[1] Over activity of rennin angiotensin system (RAS) are involved in the pathophysiology of renal and cardiovascular disease.[2] The RAS has an important role in the control of body fluid, electrolyte balance and arterial pressure.[3,4] Sex hormones, especially estrogen, are contributed to gender differences. Estrogen activates the synthesis of the rennin substrate angiotensinogen[5] and it inhibits plasma renin activity.[6] It is reported that estrogen administration in ovarectomised rats is caused upregulation of angiotensin type 2 receptor (AT2R),[7] and angiotensin II (AngII) is caused a stronger vasoconstriction in ovarectomised rats.[8]

Nitric oxide (NO) is an endogenous mediator in different biological actions including vasodilation.[9] It also was reported that NO releasing was significantly higher in ovarectomised rats treated with estradiol (OVE)[10] and estrogen induces augmentation of NO production through vascular endothelium.[11] So, with attention to role of estrogen in AT2R expression and NO releasing, in this study we attempt to find the role of estrogen on NO releasing in response to AngII in presence and absence of AT2R in female rats.

METHODS

A total of 33 female Wistar rats were used in this research study. The rats were housed at a temperature of 23-25°C with a 12 h light/dark cycle and they had free access to water rat chow. The experimental groups were as (1) intact animals; (2) ovariectomised rats treated with placebo for 2 weeks (OVX), and (3) OVE valerate (0.5 mg/kg body weight, i.m) for 2 week (OVE). Rats were anesthetized with Inactin (Sigma St. Louis USA), trachea was isolated to insert air ventilation tube and catheters were implanted into the carotid artery and jugular vein. Blood pressure was monitor through carotid artery and after the equilibration period, rats from each group were divided into two subgroups. One subgroup received saline vehicle (0.9% saline) and the other subgroup received AT2R antagonist; PD123319 (Sigma, St. Louis, MO, USA, 1 mg/kg as bolus plus continuous infusion of 1 mg/kg/h during the experiment). At 30 min after commencing vehicle or antagonists treatments, intravenous infusions AngII (20 μg/kg/h) for period of 60 min commenced. The blood samples also were obtained before and after the AngII infusion for nitrite concentration determination. The level of nitrite was measured using a colorimetric assay kit (Promega Corporation, USA) that involves the Griess reaction.

Statistical analysis

Data are expressed as mean ± SEM. The serum level of nitrite before (control) and after (treat) AngII infusion between the groups were compared via repeated measures ANOVA with factors group (intact, OVX and OVE) and time (before and after AngII infusion) and their interaction. $P \leq 0.05$ was considered to be significant.

RESULTS

Serum nitrite concentration

The results are presented in Figure 1a and b. At control phase (before AngII administration), no significant differences were observed between the subgroups neither treated with vehicle nor PD123319. After AngII administration when AT2R antagonist (PD123319) was not infused, the serum level of nitrite in response to AngII administration in OVE groups increased significantly [Figure 1a] ($P < 0.05$). However, this increase was abolished by AT2R antagonist [Figure 1b].

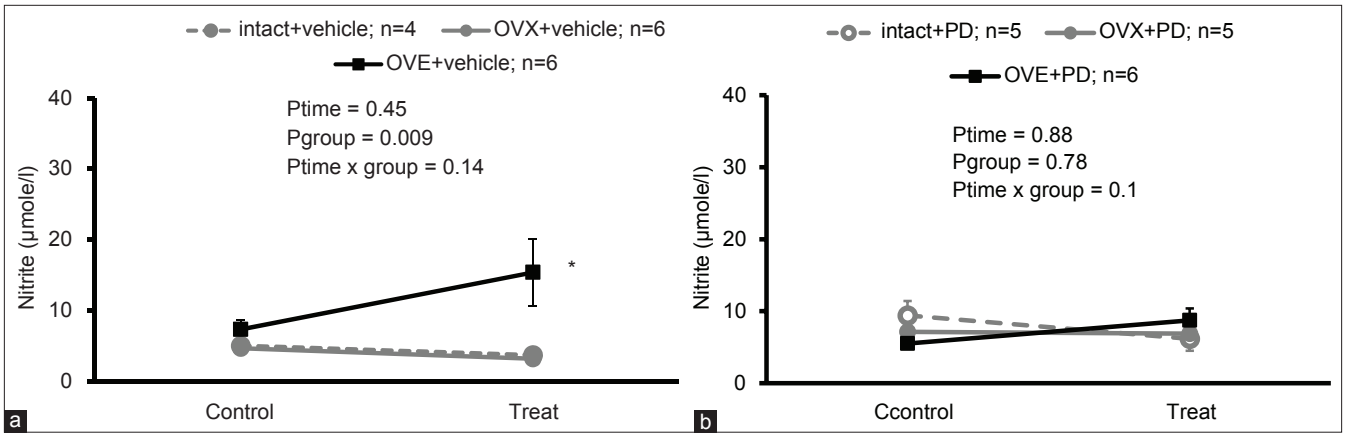

Figure 1: The serum level of nitrite in three groups of experiments before (control) and after (treat) angiotensin II administration without (a) And with (b) Antagonist; PD123319. Significant difference was observed between estradiol and other groups when angiotensin type 2 receptor was present. Star (*) indicate significant difference from others group ($P < 0.05$). OVX; ovariectomised rats treated with placebo, OVE; ovariectomised rats treated with estradiol, and n in the top legend shows the number of animal in each subgroups

DISCUSSION

In this short study, we found that the serum level of nitrite increased significantly after AngII administration in OVE group when AT2R is present, and this increase was abolished by AT2R antagonist. Estrogen is responsible of complex effects on RAS components[12] and there is interaction between estrogen and AT2R.[13] AT2R activation opposes with AT1R actions[13] and estrogen decreases AT1R/AT2R ratio.[14] Confirmed with our result other studies have reported that ovariectomised rats treated with estrogen is caused enhancing endothelium derived relaxing factor level[10] due to increasing of endothelial NO synthase. It seems that estrogen with effect on AT2R regulates its functions.[15] The vasodilatory effect of estrogen response to AngII administration may mediate by AT2R to increase the serum level of NO metabolite in female rats. Due to gender difference of AngII receptors activities,[16-18] the role of AT2R in NO formation response to AngII infusion may be different in male.

CONCLUSIONS

AT2R is involved in serum nitrite level response to AngII infusion in OVE.

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