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The Role of Mas Receptor and Bradykinin on Nitric Oxide Production Response to Angiotensin 1-7 in Ovariectomized Rats Treated with Estradiol

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Original Article

Abstract

Introduction: The angiotensin 1-7 (Ang1-7) Mas receptor (MasR) axis, bradykinin (BK) and female sex hormone are involved in releasing of vasodilatory biomarkers including Nitric Oxide (NO). We examined the role of MasR and BK on NO metabolite (nitrite) production response to Ang 1-7 infusion in ovariectomized rats treated with estradiol.

Methods: A total of 48 female Wistar rats were divided into 2 main groups; ovariectomized treated with placebo (OVX) and ovariectomized treated with estradiol valerate (OVE) for period of two weeks. Then after anesthetization, the animals of each groups were divided into four subgroups that received MasR antagonist (A779) or , BK, BK+A779 or vehicle, and they were subjected to Ang1-7 infusion (0, 100, 300 and 1000 ng/kg/min). The level of nitrite (NO metabolite) was measured by Griess method.

Results: The serum level of nitrite response to Ang 1-7 administration in OVE group was incrased when compared with OVX group, however when MasR was blocked by A799, the increased nitrite level was abolished. BK also increased the level of nitrite but co administration of BK and A779 did not enhance the nitrite level in both OVE and OVX groups.

Conclusion: Estradiol and Bk increase nitrite production in response to Ang 1-7 infusion in condition of MasR presence.

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Introduction:

It has been well known that ovarian sex steroid hormone, estrogen provides depressor effect on blood pressure, and decrease the risk of cardiovascular and renal diseases (1-3). The mechanism of estrogen-induced vasodilation is related to nitric oxide (NO) production by the vascular endothelium, and it may also contribute to influence renin-angiotensin and kallikrein-kinin system (4-6).

NO is formed from L-arginine through catalysis by NOS. Three different isoforms of NO synthase exist: endothelial NOS (eNOS or NOS III), neuronal NOS (nNOS or NOS I) and inducible

NOS (iNOS or NOS II) (7). The function of NO appear to be essential for regulation of vascular ton and blood flow (4). This peptide has different roles depending on which isoforms of NOS enzyme release it. High levels of NO are produced by iNOS under pathological conditions, however eNOS-derived NO has beneficial effects (8-10). It is known that angiotensin 1-7 (Ang1-7), Mas receptor (MasR) and bradykinin (BK) exert their functions via NO signaling (11). The Ang 1-7/Mas axis and BK are key factors in control of vascular tone. Ang1-7, as a metabolite of RAS, acts oppose the vasoconstrictory function of angiotensin II (AngII) (12). Ang 1-7 via MasR regulates vasodilatory action by activation of NO signaling (13,14). In addition, BK is a main product of action of kallikrein enzyme to kininogens. The existence of two subtypes of BK receptors, B1 and B2, has been demonstrated, and BK mediates its vasodilatory function via B2 receptor (B2R) (15).

Several studies suggest that cooperation between Ang 1-7 and BK enhances relaxation response, mediated by NO release (11,16). It also indicates that administration of A779 as a MasR antagonist, inhibits the potentiating activity of Ang 1-7 and BK suggesting that MasR is contributed the vasodilatory effect of those factors (16). Furthermore, sex-related differences in the regulation of cardiovascular function and blood pressure were observed by estrogen. Women are less prone to high blood pressure compare with men suggesting that females have protective factors (1). In addition the role of NO is increased in female subjects may be a useful new therapeutic approach in the control of hypertension (17). On the other hand, it is well known that estrogen is involved to NO production by Ang 1-7/Mas axis and BK but it seems that the synergetic interaction between Ang1-7, BK and estradiol to stimulate NO release need to further investigation. In this study we attempted to find the role of estrogen on Ang 1-7 and BK induced-NO, in the presence and absence of MasR in ovariectomized female rats.

Methods:

A total 48 female rats (200±20 gr) were housed in the animal room with controlled temperature of 23–25°C and 12h light/dark cycle with free access to water and chow. Female Wistar rats were ovariectomized as described before (5). The two main groups named OVE group (group 1) that received estradiol valerate (500 g/kg/twice weekly, im; Aboryhan, Iran) dissolved in sesame oil, and OVX group (group 2) that received vehicle (sesame oil) for period of two weeks. Then at the day of experiment, the rats were anaesthetized by urethane (1.7 g/kg i.p.; Merck, Germany) and the tracheostomy was performed to insert air ventilation tube. Jugular vein catheterization via polyethylene tubing (PE 9658, Microtube Extrusion, North Rocks NSW, Australia) was done for drug administration. Both OVE and OVX female rats were divided into four subgroups (total of 8 subgroups). The effects of a) saline as vehicle, b) A779 (Bachem, King of Prussia, MO, USA) as a MasR blockade (50μg/kg/h), c) BK (150μg/kg/h) and d) BK+ A779 during Ang 1-7 (Bachem, King of Prussia, MO, USA) infusion (0,100,300 and 1000 ng/kg/min) were examined. Drugs were administrated via infusion pump (New Era pump system Inc., Farmingdale, NY, USA). At the end of the experiment the blood samples also were obtained after those drug adminstration for nitrite concentration determination. The level of nitrite was measured using a colorimetric assay kit that involves the Griess reaction.

For statistical analysis, comparison between OVE and OVX rats was performed using the *t*-Student test. Values of $P<0.05$ were considered statistically significant.

Results:

Estradiol increased the serum level of nitrite significantly in response to Ang1-7 administration presence of MasR ($P<0.05$) (Fig 1- saline), but the enhanced serum level of nitrite was abolished when MasR was blockade by A779 (Fig1-A779).

BK also enhanced the serum level of nitrite significantly in response to Ang1-7 in OVE group when compared with OVX group (Fig-BK), however this difference was abolished when combination of BK and A779 was administrated (Fig1-BK+A779). So that in presence of Mas antagonist, A779, the enhancement of levele of

nitrie was abolished, it means MasR is involved to nitric oxide production.

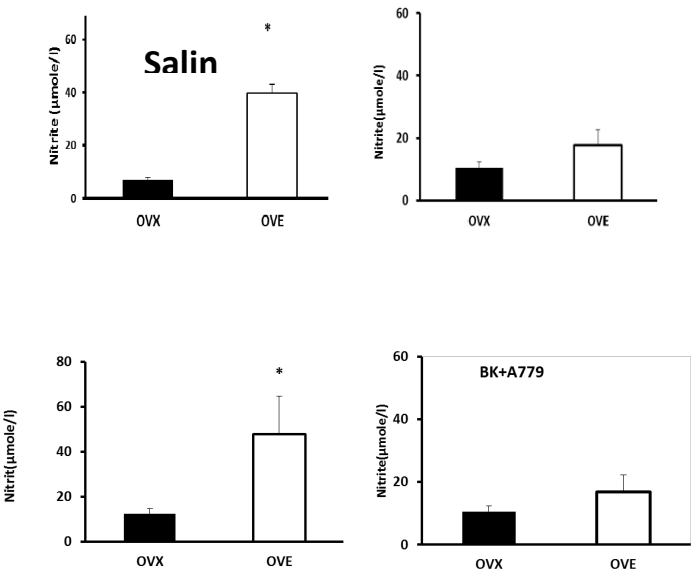

Figure 1. The serum level of nitrite in all eight groups of experiments. The nitrite level was analyzed for saline or each drug groups A779, BK, BK+A779 in response to angiotensin 1-7 administration in OVE and OVX rats. Star (*) indicate significant difference between OVE and OVX groups (P<0.05). OVX; ovariectomised rats treated with placebo, OVE; ovariectomised rats treated with estradiol. BK; Bradykinin, A779; Mas receptor antagonist.

Conclusion:

In this study, it is found that different dose of Ang 1-7 increased significantly the level of nitrite in OVE group compare with OVX group, but this difference between two groups was abolished by MasR antagonist (A779). In addition, estradiol enhanced the level of nitrite in response to Ang 1-7 when BK was present but a combination of BK and A779 decreased this enhancement. Therefore when MasR was blocked, the estradiol and Ang1-7 cannot increase the level of nitrite whether BK was present or not.

The importance role of estrogen for regulating NO pathway has been demonstrated before (18-20).

In condition of estrogen-deficient, the endothelial function is decreased via decreasing of NO and endothelium-dependent hyperpolarization production (18,21,22). Caliman et al showed that estrogen deficiency leads to endothelial dysfunction in female rats with iNOS overexpression and reduced eNOS expression (19). It is known that measurement of nitrite level in blood reflects amount of endothelial NO production (23). It is reported that estradiol enhanced circulating nitrite levels in follicular phase and this effect don't correlated with levels of progesterone (20). In addition, this hormone augmented the level of nitrite and nitrate in aortic endothelial cells (24). Our results are in accordance with the findings of those studies confirm that steroid hormone enhance the plasma level of nitrite

It is known that estrogen increases NO release by enhancement NO synthase activity, NO synthase protein, and the essential cofactors for NO synthase (20). On the other hand, estrogen induces the vasodilator response to BK and Ang 1-7 by NO production (4,5). It is also well documented that estrogen up regulates AngII type 2 receptor (AT2R), MasR and B2R which activate depressor pathways so that the female sex hormone exert vosodilatory response by Mas and B2 receptors (25-28). Ang 1-7/Mas axis of RAS mediates its functions by prostanoids, NO production. It also seems that cooperation between BK and Ang 1-7 leads to the vasodilatory effect that is mediated by MasR (16). In current study, estradiol contributes to elevate serum of nitrite and the mechanism of this effect may be mediated by Ang1-7/mas axis and BK pathway.

Prior study reported that vasodilator effect of Ang1-7 in rabbit afferent arterioles is mediated by NO pathway but MasR antagonist A779, inhibited depressor B2 receptor action of this peptide (29). In addition, Bk as a key mediator of kallikrein-kinin system diminish blood pressure via its B2R (15). In regard to intraction between Mas and B2 receptors, inhibition of B2R by HOE-140 blockade depressor action of Ang1-7/Mas axisis (30) and also MasR are involved to synergistic influence of BK on Ang1-7 action (16). Additionally, L-NAME as a non-selective NO synthase inhibitor, attenuates the relaxation effects of Ang1-7 and BK suggestiong the role of NO signaling (31). According to the above sentences may be the response of different dose of Ang1-7 to NO production is depends on interaction between Mas and BK receptors.

Our current study has a number of limitations. Firstly, about estrogen therapy method, it would be better to use estrogen pellets instead of IM (Intramuscular injection) injection. Secondly, we did not assess blood NO before drug administration because multiple blood sampling, decrease blood pressure during experiment.

It is concluded that MasR has a central role in NO-release by cooperation effect between estradiol, Ang 1-7 and BK. Therefore when MasR was blocked the enhancement of the levelel of nitrie, as index of NO production, was abolished.

Confilict of interest:

The authors declare no conflict of interest.

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