

# 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 increased 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.

**Key words:** Angiotensin 1-7, Mas Receptor, Nitric Oxide, Bradykinin, Rat

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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 administration for nitrite concentration determination. The level of nitrite was measured using a colorimetric assay kit that involves the Griess reaction.

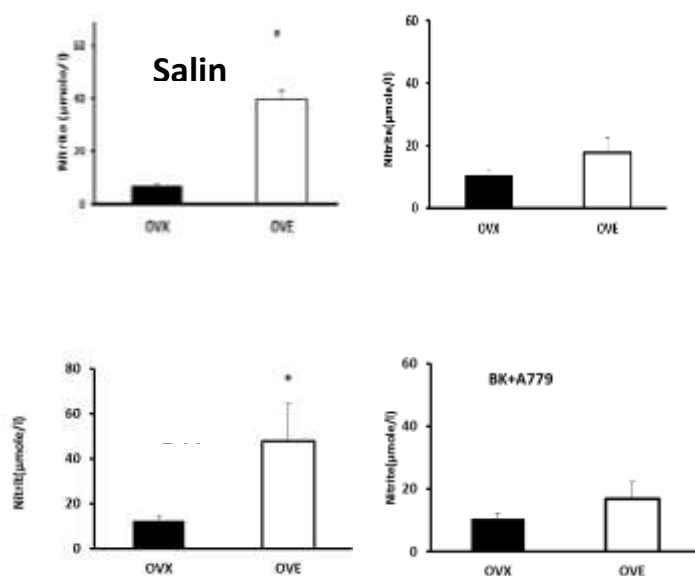
For statistical analysis, comparison between OVE and OVX rats was performed using the *t*-Student test. Values of  $P < 0.05$  were considered statistically significant.

## 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

nitrite 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 vasodilatory 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 interaction between Mas and B2 receptors, inhibition of B2R by HOE-140 blockade depressor action of Ang1-7/Mas axis (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 suggesting 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 level of nitric oxide, as index of NO production, was abolished.

### Conflict of interest:

The authors declare no conflict of interest.

### References:

- Gouva L, Tsatsoulis A. The role of estrogens in cardiovascular disease in the aftermath of clinical trials. *hormones-athens*. 2004;3(3):171-183.
- Chappell MC, Marshall AC, Alzayadneh EM, Shaltout HA, Diz DI. Update on the angiotensin converting enzyme 2-angiotensin (1-7)-Mas receptor axis: fetal programming, sex differences, and intracellular pathways. *Front Endocrinol (Lausanne)*. 2014;4:201.
- Seliger SL, Davis C, Stehman-Breen C. Gender and the progression of renal disease. *Curr Opin Nephrol Hypertens*. 2001;10(2):219-225.
- Dehghani A, Saberi S, Nematbakhsh M. Nitric Oxide Synthases Blockade; L-NAME and Estradiol Alter Renal Blood Flow Response to Angiotensin 1-7 in Ovariectomized Rats. *Indian J Physiol Pharmacol*. 2015;59(3); 266-274.
- Dehghani A, Saberi S, Nematbakhsh M. Role of Mas receptor antagonist A799 in renal blood flow response to Ang 1-7 after bradykinin administration in ovariectomized estradiol-treated rats. *Adv Pharmacol Sci*. 2015; 2015:801053.
- Saberi S, Dehghani A, Nematbakhsh M. Role of Mas receptor in renal blood flow response to angiotensin-(1-7) in ovariectomized estradiol treated rats. *Res Pharm Sci*. 2016;11(1):65-72.
- Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J*. 2012;33(7):829-837.
- Aktan F. iNOS-mediated nitric oxide production and its regulation. *Life Sci*. 2004;75(6):639-653.
- Fedorov R, Vasani R, Ghosh DK, Schlichting I. Structures of nitric oxide synthase isoforms complexed with the inhibitor AR-R17477 suggest a rational basis for specificity and inhibitor design. *Proc Acad Sci USA*. 2004;101(16):5892-5897.
- Nematbakhsh M, Pezeshki Z, Jazi FE, Mazaheri B, Moeini M, Safari T, et al. Cisplatin-Induced Nephrotoxicity; Protective Supplements and Gender Differences. *Asian Pac J Cancer Prev: APJCP*. 2017;18(2):295-314.
- Brosnihan KB, Li P, Ferrario CM. Angiotensin-(1-7) dilates canine coronary arteries through kinins and nitric oxide. *Hypertension*. 1996;27(3 pt 2):523-528.
- Zhu Z, Zhong J, Zhu S, Liu D, van der Giet M, Tepel M. Angiotensin-(1-7) Inhibits Angiotensin II-Induced Signal Transduction. *J Cardiovasc Pharmacol*. 2002;40(5):693-700.
- Ferrario CM, Chappell MC, Tallant EA, Brosnihan KB, Diz DI. Counterregulatory actions of angiotensin-(1-7). *Hypertension*. 1997;30(3 pt 2):535-541.
- Santos RA, e Silva ACS, Maric C, Silva DM, Machado RP, de Bühr I, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proceedings of the PNAS*. 2003;100(14):8258-8263.
- Bhoola K, Figueroa C, Worthy K. Bioregulation of kinins: kallikreins, kininogens, and kininases. *Pharmacol Rev*. 1992;44(1):1-80.
- Oliveira MA, Fortes ZB, Santos RA, Kosla MC, De Carvalho MHC. Synergistic effect of angiotensin-(1-7) on bradykinin arteriolar dilation in vivo. *Peptides*. 1999;20(10):1195-1201.
- Besco R, Sureda A, Tur JA, Pons A. The effect of nitric-oxide-related supplements on human performance. *Sports Medicine*. 2012;42(2):99-117.
- Nawate S, Fukao M, Sakuma I, Soma T, Nagai K, Takikawa O, et al. Reciprocal changes in

- endothelium-derived hyperpolarizing factor-and nitric oxide-system in the mesenteric artery of adult female rats following ovariectomy. *Br J Pharmacol.* 2005;144(2):178-189.
19. Caliman IF, Lamas AZ, Dalpiaz PLM, Medeiros ARS, Abreu GR, Figueiredo SG, et al. Endothelial relaxation mechanisms and oxidative stress are restored by atorvastatin therapy in ovariectomized rats. *PLoS One.* 2013;8(11):e80892.
  20. Rosselli M, Imthurn B, Keller PJ, Jackson EK, Dubey RK. Circulating nitric oxide (nitrite / nitrate) levels in postmenopausal women substituted with 17 $\beta$ -estradiol and norethisterone acetate. *Hypertension.* 1995;25(4 pt 2):848-853.
  21. Ge Z-D, Zhang X-H, Chin-Wan Fung P, He G-W. Endothelium-dependent hyperpolarization and relaxation resistance to N G-nitro-l-arginine and indomethacin in coronary circulation. *Cardiovascular Research.* 2000;46(3):547-556.
  22. Sakuma I, Liu MY, Sato A, Hayashi T, Iguchi A, Kitabatake A, et al. Endothelium-dependent hyperpolarization and relaxation in mesenteric arteries of middle-aged rats: influence of oestrogen. *Br J Pharmacol.* 2002;135(1):48-54.
  23. Lauer T, Preik M, Rassaf T, Strauer BE, Deussen A, Feelisch M, et al. Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. *PNAS.* 2001;98(22):12814-12819.
  24. Hishikawa K, Nakaki T, Marumo T, Suzuki H, Kato R, Saruta T, editors. Induction of constitutive nitric-oxide synthase by estradiol in human endothelial-cells. *Hypertension;* 1994; Sep 1 (Vol.24, NO 3, PP. 386). 7272
  - greenville avenue, dallas, tx 75231-4596: Amer Heart Assoc.
  25. Sampson AK, Moritz KM, Denton KM. Postnatal ontogeny of angiotensin receptors and ACE2 in male and female rats. *Gend Med.* 2012;9(1):21-32.
  26. Brosnihan KB, Li P, Ganten D, Ferrario CM. Estrogen protects transgenic hypertensive rats by shifting the vasoconstrictor-vasodilator balance of RAS. *Am J Physiol-Regula, Integ Comp Physiol.* 1997;273(6):R1908-R15.
  27. Veille JC, Li P, Eisenach JC, Massmann AG, Figueroa JP. Effects of estrogen on nitric oxide biosynthesis and vasorelaxant activity in sheep uterine and renal arteries in vitro. *Am J Obstet Gynecol.* 1996;174(3):1043-1049.
  28. Weiner CP, Lizasoain I, Baylis SA, Knowles RG, Charles IG, Moncada S. Induction of calcium-dependent nitric oxide synthases by sex hormones. *PNAS.* 1994;91(11):5212-5216.
  29. Ren Y, Garvin JL, Carretero OA. Vasodilator action of angiotensin-(1-7) on isolated rabbit afferent arterioles. *Hypertension.* 2002; 39(3):799-802.
  30. Abbas A, Gorelik G, Carbini L, Scicli A. Angiotensin-(1-7) induces bradykinin-mediated hypotensive responses in anesthetized rats. *Hypertension.* 1997;30(2):217-221.
  31. Peiró C, Vallejo S, Gembardt F, Palacios E, Novella S, Azcutia V, et al. Complete blockade of the vasorelaxant effects of angiotensin-(1-7) and bradykinin in murine microvessels by antagonists of the receptor Mas. *J Physiol.* 2013;591(9):2275-2285.

## بررسی نقش آنتاگونیست گیرنده Mas و برادی کینین در پاسخ به آنژیوتانسین ۱-۷ بر غلظت نیتريت سرم در رت های اورکتومی شده دریافت کننده استروژن

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### چکیده

**مقدمه:** محور وازودیلاتوری سیستم رنین آنژیوتانسین (RAS) (آنژیوتانسین ۱-۷ و گیرنده Mas) و برادی کینین همراه با هورمون جنسی زنانه، استروژن، نقش اساسی در پیشبرد پاسخ وازودیلاتوری و کاهش فشارخون دارند. در این مطالعه، نقش محور آنژیوتانسین ۱-۷ گیرنده Mas و برادی کینین بر تولید نیتريك اكساید در رت های اورکتومی شده دریافت کننده استروژن بررسی شد.

**روش کار:** ۴۸ سر رت ماده نژاد ویستار تخمدان برداری شده، به طور تصادفی به دو گروه اصلی دریافت کننده استرادیول والرات (OVE) و (OVX) Vehicle تقسیم شدند. بعد از دو هفته از تزریق حیوانات بی هوش شده و به چهار زیرگروه دریافت کننده (۱) آنتاگونیست گیرنده Mas (۲) برادی کینین، (۳) آنتاگونیست گیرنده MAS + برادی کینین، (۴) سالین طبقه بندی شدند و سپس هر گروه از حیوانات تحت انفوزیون آنژیوتانسین ۱-۷ با دوزهای ۰، ۰، ۱۰۰، ۲۰۰، ۳۰۰، ۱۰۰۰ به ازای هر کیلوگرم وزن بدن در دقیقه قرار گرفتند. در پایان هر آزمایش خونگیری جهت تعیین سطح سرمی نیتريت (متابولیت نیتريك اكساید) توسط متد گریس انجام شد.

**نتایج:** با انفوزیون آنژیوتانسین ۱-۷ سطح نیتريت سرم در رت های OVE در مقایسه با گروه OVX به طور معنی داری افزایش یافت اما در شرایطی که گیرنده Mas بلوک شد تفاوتی در دو گروه OVX و OVE مشاهده نشد. از طرفی، استروژن در حضور برادی کینین سطح نیتريت سرم خون را در پاسخ به آنژیوتانسین ۱-۷ افزایش داد اما با تزریق برادی کینین همراه با A779 این پاسخ افزایشی ایجاد شده توسط استروژن مشاهده نشد.

**نتیجه گیری:** استروژن و برادی کینین در پاسخ آنژیوتانسین ۱-۷ موجب افزایش سطح نیتريك اكساید در حضور گیرنده Mas شدند.

**کلیدواژه ها:** آنژیوتانسین ۱-۷، گیرنده Mas، نیتريك اكساید، برادی کینین، رت

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