Angiotensin 1-7 Receptor and Angiotensin II Receptor 2 Blockades Prevent the Increased Serum and Kidney Nitric Oxide Levels in Response to Angiotensin II Administration: Gender-Related Difference

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ABSTRACT

Background: The angiotensin II (Ang II) receptor 2 (AT2R) and angiotensin 1-7 receptor (masR) expression in the kidney are gender-related. We attempted to compare the response of nitric oxide (NO) production to Ang II administration, with and without AT2R and masR blockades, using A-779 and PD123319 in male and female rats.

Methods: Anesthetized and catheterized male and female Wistar rats were subjected to one-hour continuous infusion of Ang II (~20 μg/kg/hour), with and without masR and AT2R blockades. The level of the NO metabolite (nitrite) was measured before and after the experiment in rat serum and in the homogenized kidney tissue.

Results: The basal data indicated that no sex difference in the serum level of nitrite could be detected before Ang II infusion. However, administration of Ang II in male and female rats caused a gender difference in the nitrite level, which resulted in the serum level of the nitrite significantly increasing in males ($P < 0.05$) when compared with the females. In addition, masR blockade or co-blockade of masR and AT2R in male rats abolished the gender difference related to the effect of Ang II on nitrite production. In the presence of masR and AT2R, or when masR alone was blocked, the level of nitrite in the kidney, in response to the Ang II infusion was not significantly different between the two sexes. On the contrary, masR and AT2R co-blockades significantly decreased the kidney nitrite concentration response to Ang II administration in both male and female rats ($P < 0.05$), but no sex difference was detected.

Conclusions: The renal vasculature of male rats may provide more response to Ang II administration-induced NO, which is dependent on masR and AT2R. During dual masR + AT2R blockades, the kidney NO formation wasreduced in a non-gender related manner.

Keywords: Angiotensin II receptor 2, angiotensin II, angiotensin1-7 receptor, nitric oxide, rat

Original Article

INTRODUCTION

The renin-angiotensin system (RAS) has an important role in modulating kidney function and blood pressure. Overactivity of

RAS has been involved in the pathophysiology of diseases, such as, heart failure, myocardial infarction, and hypertension.[1] The angiotensin-converting enzyme (ACE) hydrolyzes Ang I to Angiotensin II (Ang II).[2] The active homolog of ACE, angiotensin-converting enzyme2 (ACE2), degrades Ang II to Ang1-7 and Ang I to Ang1-9.[3]

There are two AngII receptors; AT1R and AT2R, and one receptor for Ang1-7 called the mas receptor (mas R). AT1R activation elicits some biological functions, such as, sodium retention, vasoconstriction, a decreased glomerular filtration rate, increased mesangial cell hypertrophy, and renal injury.[4,5] AT2R and masR antagonize the effects of AT1R via nitric oxide (NO) formation;[6] thus leading to vasodilation, which improves the renal blood flow and enhances pressure natriuresis.[7,8] Ang1-7 stimulates NO and prostaglandin production[9] and it is the physiological antagonist for Ang II.[10] Estradiol increases the vasodilator effect of Ang1-7[11] and there is a gender difference in the vascular response to Ang II and AT2R expression.[4]

Nitric oxide is formed from L-arginine by NO synthase, and it is important for the control of renal vascular tone and renal hemodynamics.[12] The renal vascular resistance is increased by NO synthase inhibition, which results in hypertension and decreased renal plasma flow.[13,14] Kidney diseases in male patients are accompanied by reduction of NO.[15,16] Considering NO and gender, the female sex hormones stimulate NO production.[17,18] In this regard, the NO synthase activity and the NO-releasing response of acetylcholine is higher in females,[19] and the tissue response to Ang II administration is attenuated by female sex hormones.[20] In addition, estradiol administration enhances the vasodilator effects of Ang1-7.[11] On the other hand, the kidney vessels in males are more dependent on NO than in females.[21-25] Accordingly, it seems that Ang II-induced NO in the kidney may be related to gender. Therefore, we hypothesized that Ang II-induced NO is gender-related and dependent on mas R or AT2R. We also attempted to find the role of gender on Ang II-induced NO in the kidney, in the presence and absence of masR, or a combination of masR and AT2R in male and female rats.

METHODS

Male and female Wistar rats were used in this research study. The rats were housed at a temperature of 23-25°C with a 12-hour light/dark cycle, and they had free access to water and rat chow. The rats were anaesthetized with Inactin (Sigma, St. Louis, USA), and the trachea was isolated to insert a air ventilation tube. A catheter was implanted into the jugular vein.

In both male and female rats, the effects of (a) a saline vehicle and (b) masR or (c) AT2R + masR blockade were tested. A779 (Bachem, King of Prussia, MO, USA) and PD123319 (Sigma, St. Louis, MO, USA) were used to block masR and AT2R, respectively. After the equilibration period, the rats received either a saline vehicle (0.9% saline; 1 ml bolus plus 1 ml/hour — group I) or A779 (50 μg kg^{-1} plus 50 μg kg^{-1} h^{-1} from a stock of 20 μg/ml — group II) or A779 plus PD123319 (1 mg kg^{-1} plus 1 mg kg^{-1} h^{-1} from a stock of 0.5 mg/ml — group III). The antagonist and saline infusions continued during the experiment. Thirty minutes after commencing the vehicle or antagonist treatments, intravenous (via jugular vein) infusions of Ang II (~20μg/kg in 60 minutes) were administered. The rats were sacrificed by the end of the experiments, and the kidneys were weighed, homogenized (in 2 ml of saline), and centrifuged; and the supernatant was collected for nitrite (stable NO metabolite) measurement. The blood samples were also obtained before and after the experiment for determination of the nitrite concentration.

The level of nitrite was measured using a colorimetric assay kit (Promega Corporation, USA) that involved the Griess reaction. Briefly, after adding sulfanilamide solution and after incubation, N-(1-Naphthyl) ethylenediamine solution was added. Next, the absorbance in the samples was measured by a microreader in the wavelength of 540 nm. The nitrite concentration of samples was determined by comparison with the nitrite standard reference curve.

Statistical analysis

Data were expressed as mean ± SEM. For the antagonists or vehicle, the comparison between male and female rats was performed using the unpaired *t*-Student test. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Effect of mas R or AT2R blockades on serum nitrite concentration

With regard to the baseline data, no significant

difference was observed in the serum nitrite level between male and female rats in each group [Figure 1]. However, after Ang II infusion, the serum level of nitrite increased significantly in males ($P < 0.05$), and a significant difference was observed between the two genders (male, 8.31 ± 2.05; female; 4.62 ± 0.68 μmole/l, $P < 0.05$). The masR or masR plus AT2R blockades abolished the Ang II-induced NO [Figure 1].

Effect of masR or AT2R blockades on kidney nitrite concentration

In the presence of masR and AT2R and also when masR was blockaded, the level of nitrite in the kidney in response to Ang II was not significantly different in male and female rats. However, a significant reduction was observed in the kidney tissue nitrite level in both sexes when the masR and AT2R were blocked [Figure 2].

DISCUSSION

In this study, we have made three observations. (1) The serum nitrite level, after Ang II administration, in the vehicle group, increased significantly in males, but not in females. The difference was abolished by masR or combination of masR and AT2R blockades. (2) In the presence of the masR blockade, the kidney nitrite concentration response to Ang II infusion did not change significantly in both sexes, as compared to the control group, however, (3) the kidney nitrite level responses to Ang II administration in male and female rats was reduced significantly by the co-blockading of masR and AT2R.

The response to Ang II has been reported to be gender-related. In an earlier study, it has been shown that the number of AT1R is higher in males than in females, which is related to the effect of estradiol.[26] Other reports indicate that blood pressure elevation in response to chronic administration of Ang II occurs more often in males,[27-29] and this response is probably related to the presence of testosterone.[30] In addition, by increasing the cGMP levels, Ang II enhances NO production.[31] Reckelhoff *et al*. have reported that the mRNA and protein levels of eNOS are higher in the kidneys of females, but the functional response to NO synthase inhibition is more prominent in the kidneys of males,[22] and increasing of the serum level of NO in response to Ang II may relate to vasoconstriction, which activates eNOS and cyclooxygenase pathways.[32,33] Accordingly, due to gender-related Ang II response, which is higher in males and a direct relationship between Ang II and NO formation via increasing cGMP, a higher Ang II-induced NO in males can be expected.

Our findings supported this expectation and Ang II-induced NO was higher in males. This sex difference was abolished by the masR blockade or by co-blockading of masR and AT2R, which suggested the important meditative role of masR for Ang II-induced NO in males; but not in females.

The AT2R expression in females is higher,[6,34] and masR or AT2R stimulation may both promote NO production,[31,32,35,36] and administration of PD123319 inhibits cGMP production and decreases NO formation.[31] Therefore, the blockade of the receptors possibly reduces NO production by different mechanisms. However, this phenomenon is more effective in males than in females because of the higher response to Ang II by males. Therefore, due to the functional role of RAS and its receptors in the kidney function and renal circulation, which is reported to be gender-related,[37-39] our finding may be evidenced for the importance of RAS receptors in Ang II-induced NO formation.

With regard to kidney NO, no sex difference was obtained, and a significant reduction in kidney nitrite level was detected during dual masR + AT2R

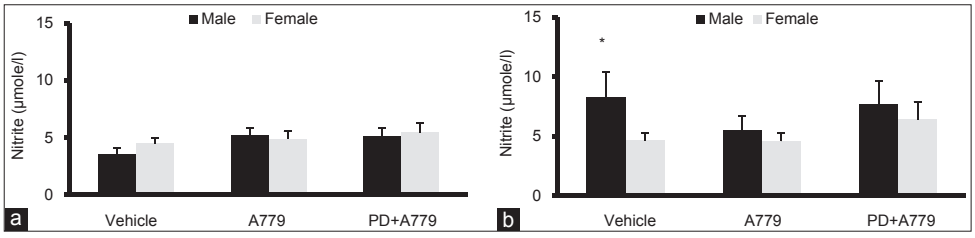

Figure 1: The serum level of the nitrite in the three experiment groups before and after angiotensin II administration. A significant difference was observed in the vehicle-treated males ($P < 0.05$)

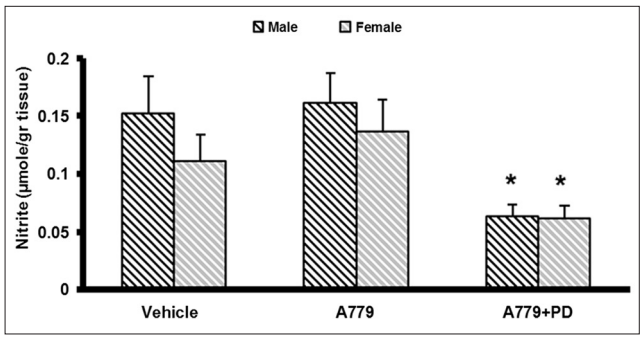

Figure 2: The kidney level of nitrite in the three experiment groups. A significant difference was observed between A779 + PD and the other groups ($P < 0.05$)

blockades in both sexes. This finding suggested the important role of AT2R in Ang II-induced kidney NO in both sexes, in spite of the higher expression of AT2R in females. However, this paradoxical response also suggested a complex interaction between masR and AT2R

CONCLUSIONS

It seems that masR involves Ang II-induced NO more in males. However, during dual masR + AT2R blockades, kidney NO formation in response to Ang II administration reduced in a non-gender-related manner.

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