INTRODUCTION

Clinical and epidemiologic evidences suggest the development and progression of renal and cardiovascular diseases are more slowly in women before menopause in comparison with men (1, 2). However, the mechanisms underlying this difference remain unknown, but renin angiotensin system (RAS) has been known to involve in this gender-based difference (3, 4). RAS plays an important role in regulation of arterial pressure by the kidney due to its effects on vascular tone and renal excretion (5). The activity and expression of RAS components and response to RAS receptor blockers and stimulators also have been reported to be gender-related (6, 7).

One of the main important components of RAS is angiotensin 1-7 (Ang1-7) and its receptor and enzyme function targeting. Blockade of angiotensin II (AngII) receptors type 1 and 2 (AT1R and AT2R) inhibits some actions of Ang1-7. We described the role of Ang1-7 receptor (MasR) antagonist (A779) on kidney hemodynamics when AT1R and AT2R are blocked with losartan and PD123319. In anaesthetized male and female rats after blockade of both AT1R and AT2R, the renal perfusion pressure (RPP) was controlled in two levels of 80 and 100 mmHg via an adjustable clamp placed around the aorta above the level of the renal arteries. Then, the effects of saline vehicle and MasR blocker (A779) were tested on pressure natriuresis and diuresis, renal blood flow (RBF), and renal vascular resistance (RVR). In the absence of AT1R and AT2R; RVR, RBF/wet kidney tissue weight, and serum level of renin did not alter in both genders either MasR was blocked or not. However, urine flow rate (UF) and sodium excretion (U NaV) increased significantly at the pressure level of 100 mmHg in the presence of MasR in male (P<0.05) but not in female rats. When AT1R and AT2R were blocked, the impact of MasR is gender-related in pressure natriuresis and diuresis, and pressure natriuresis and diuresis in male rats (not female) increases in the presence of MasR.

Key words: angiotensin 1-7, Mas receptor, angiotensin II receptors, angiotensin converting enzyme, renal blood flow, urine output, renin, sodium excretion
MasR may modulate the natriuresis relationship, contributing to gender-associated differences in blood pressure regulation (22). Accordingly, in this study, we examined the role of MasR in pressure-natriuresis in male and female rats when AT1R and AT2R were blocked.

MATERIALS AND METHODS

Animal

The experimental procedures were approved in advance by the Isfahan University of Medical Sciences Ethics Committee. Male (n = 28, 190 ± 2.3 g) and female (n = 28, 180 ± 1.0 g) Wistar rats (Animal Centre, Isfahan University of Medical Sciences) were used. The rats were individually housed at a temperature of 23–25°C with a 12-h light/dark cycle, with the dark period between 19.00–07.00.

Surgery

The rats were anaesthetised (Inactin; thiobutabarbital, 175 mg kg\(^{-1}\) i.p. Sigma, St Louis, MO, USA.) and the trachea was isolated to insert air ventilation tube. Catheters were implanted into the jugular vein, and carotid and femoral arteries. A catheter was also inserted in the bladder to collect the urine volume. An adjustable clamp was placed around the aorta above the level of the renal arteries to control renal perfusion pressure (RPP). RPP was measured from the femoral artery. The left kidney was placed in a stable cup. The flow probe was placed and fixed around left renal artery, and renal blood flow (RBF) was monitored by transit-time ultrasound flowmetry (Transonic Systems, Ithaca, NY, USA.). Body temperature was continuously monitored through the experiment. We allowed 30–60 minutes for equilibration period.

Experimental protocol

After equilibration period, male and female rats in treated groups simultaneously received MasR (A779), AT1R (losartan), and AT2R (PD123319) blockers via jugular vein by micro-infusion pumps until the end of the study. Losartan, PD123319, and A779 were purchased from Darou Pakhsh Pharma Co. (Tehran, Iran), Sigma (St. Louis MO, USA), and Bachem Bioscience Inc. (King of Prussia, PA, USA), respectively. Male and female rats in control groups had the same treatment except vehicle instead of MasR blocker. Blood sample was collected at the end of the experiment via catheter line. Urinary sodium concentration was measured using Convergys ISE Analyzer. The serum level of renin was measured using a rat renin ELISA kit (Glory Science Co., Ltd, USA). Finally, the animals were sacrificed humanely; the left kidney was removed immediately and weighed. Renal vascular resistance (RVR) was calculated as RPP/RBF ratio.

Statistical analysis

Data are expressed as mean ± S.E.M. Unpaired t-test was used to compare each parameter between the groups. Values of P<0.05 were considered statistically significant.

RESULTS

The role of MasR on urine parameter in both genders with two different RPP when AT1R and AT2R were blocked

There was an increase in urinary sodium excretion (\(U_{\text{NaV}}\); P<0.05), urine flow (UF; P<0.05) and urine weight (P<0.05) from low to high RPP in the male rats. Such observation was not seen in female rats. These data indicated that MasR is involved in pressure natriuresis and diuresis in male but not in female when AT1R and AT2R were absent. In other words, AT1R and AT2R blockade could not abolish pressure natriuresis and diuresis in male rats (Fig. 1).

The role of MasR on RBF, RVR and serum level of renin in both genders with two different RPP when AT1R and AT2R were blocked

In both genders; RBF, RVR, and renin concentration did not differ between two levels of RPPs in both sexes. The data indicated that MasR blockade (by A779) abolished the effect of RPP on RBF, RVR and renin concentration when angiotensin II receptors are blocked (Fig. 2).

DISCUSSION

There were two major findings in this study when AT1R and AT2R were blocked. Firstly, it was shown that the impact of MasR on pressure natriuresis and diuresis is gender-related. Secondly, in both genders, RBF/wet kidney tissue weight and RVR did not alter by increased pressure in the presence or absence MasR.

Sexual differences in blood pressure are associated with the angiotensin converting enzyme 2 (ACE2)/Ang 1-7/MasR axis, while the effects of ACE and AT1R blockers partly depend on ACE2 and Ang 1-7 (25). It was demonstrated that ACE2 activity and Ang 1-7 levels increased (5-25-fold) in male rats treated with an ACE inhibitor or AT1R blockers and blood pressure also enhanced by blocking Ang 1-7 synthesis (3, 26-29). Also, previous studies have shown that administration of A779 or an ACE2 inhibitor was accompanied by worsening of renal function and hypertension (25). Therefore, it could be concluded that Ang 1-7 is influenced by antihypertensive and renal protective actions of ACE inhibition and AT1R blocker (30).

Drugs which influence RAS axis may inhibit Ang II production and activate Ang 1-7 formation (5). In this regard, it has been showed that aortic tissue of apoE knockout mice may generate AngII which could be attenuated by AVE0991; Ang 1-7 peptidomimetic (10). In the current study and in the presence of
MasR, the effect of pressure-dependent increase in UF and UNaV was not observed in female rats. This observation could be related to genetic polymorphisms in ACE2 in women. The genetic polymorphisms in ACE2 in women attenuate the blood pressure-lowering effect of the ACE inhibitor, while polymorphisms in ACE may influence the effectiveness of ACE inhibition and AT1R blocker in men (31, 32). Thus, it seems that Ang 1-7 contributes to the antihypertensive action of ACE inhibition and AT1R blocker treatment via MasR (33-35).

The MasR is expressed in renal proximal tubular cells, afferent arterioles, cardiac myocytes, and neuronal cells; and it conveys Ang 1-7 signals via transcriptional factors (33). It has been shown that activation of the non-classical pathway (Ang 1-7/AT2/MasR) stimulates NO and prostaglandin production that
causes vasodilation, and improves the renal blood flow and also enhances pressure natriuresis (30, 36). Therefore, endogenous Ang 1-7 and/or an Ang 1-7-related peptide exert a vasodilator effect through MasR or through potentiation of bradykinin (15, 37). Ang 1-7 can bind to ACE that facilitates the crosstalk between ACE and bradykinin B2 receptor, and this phenomenon leads to inhibition of the ACE C-domain (38, 39). In this regard, Ang 1-7 acts as an ACE inhibitor to potentiate the vasodilator actions of bradykinin through release of vasodilators, prostacyclins, NO, or endothelium-derived hyperpolarizing factor (15, 38, 40). The ACE polymorphisms in male is probably the reason for effectiveness of bradykinin potentiation by Ang 1-

Fig. 2. Renal blood flow (RBF), renal vascular resistance (RVR), and serum renin concentration for two different renal perfusion pressures (RPP) in male and female rats treated with MasR blocker (A779) when both AT1R and AT2R were blocked with losartan and PD123319. RPP80 and RPP100 stand for RPP of 82–85 and 97–103 mmHg, respectively. Data are presented as mean ± S.E.M. and were analyzed using unpaired t-test between two RPPs n=5–10 per group.
7 (32). Thus, the effect of pressure natriuresis will be seen in the presence of MasR. Also, potentiation of vasodilator effects of bradykinin by Ang 1-7 enhances natriuresis and diuresis by increasing the elimination of water and sodium from the organism. This effect is mediated by an Ang 1-7 receptor (41).

In spite of extensive studies, role of Ang 1-7 in renal physiology is not still obvious and sometimes is contrary (42, 43). The diuretic/natriuretic actions of Ang 1-7 have been explained in in vitro and in vivo experimental models (20, 21). Moreover, some studies have shown an antidiuretic/antiatriuretic effect made by Ang 1-7 (44, 45). These differences can be related to sodium and water situation, Ang 1-7 concentration, or experimental condition. Furthermore Ang 1-7 effects are related to some factors such as the nephron segment, the local expression of Ang 1-7, and its receptors (46-49). Garcia and Garvin explained that Ang 1-7 at physiologic and supra physiologic doses differently act in the kidney (50). Augmented circulating levels of Ang 1-7 in transgenic rats (TGR [A1-7] 3299) caused an antidiuretic effect, which was not related to vasopressin release (51). In pregnant rats, Joyner et al. reported that Ang 1-7 induces diuresis while in virgin females this heptapeptide causes antidiuresis (52). There are several reports consistent with our study that shows a natriuretic/diuretic action of Ang 1-7. For instance, Handa et al. explained that in anesthetized rats, administration of Ang 1-7 increases urinary flow rate and sodium excretion, while these effects were abolished by A779 (20). Also, Ang 1-7 seems to be a potent inhibitor of Na-K-ATPase activation in the renal cortex and in isolated proximal convoluted tubules (53). In renal tubular epithelial cells, Ang 1-7 inhibited trans cellular flux of sodium (21). Most studies indicate that although most Ang 1-7 effects seem to be mediated by MasR activation, this heptapeptide can also interact with AT1R, AT2R, bradykinin B2, and vasopressin V2 receptors (8, 45, 48, 54, 55).

In our study, RBF/wet kidney tissue weight, RVR, and the serum level of renin in both genders did not alter by increased pressure in the presence or absence of MasR.

Previous studies have supported contribution of the MasR to regulation of renal function by measuring the renal expression in both the tubular and vascular elements of the kidney (57). MasR mRNA expression was detected in rat cortical and proximal tubular cells, cardiac myocytes and neuronal cells (33, 58), as well as mouse afferent arterioles and tubular epithelium (59). AT1R and AT2R antagonists can involve in the Ang 1-7 effects at renal level by making oligomers including Mas-Mas sensitive to AT1R or AT2R antagonist or AT1-Mas, AT2-Mas, or AT1-AT2-Mas oligomers or cross talk (60-62). These cross talks may involve in kidney autoregulation controlling RBF, RVR, and renin concentration in the presence or absence of MasR in males and females.

Our study is partly consistent with the findings reported by Safari et al. They showed that co-administration of PD123319 and A779 eliminated the effect of A779 on RBF and RVR, and they suggested that to achieve complete renal vasoconstriction effects of MasR blockade, co-activation of AT2R is required (57). Possibly the crosstalk between the AT1R, AT2R, MasR, and bradykinin receptors, which have been reported to form heterodimers, provides a situation under which expression and activity of receptors may occur differently (9, 11, 57, 63, 64). Another possibility is that the signal transduction pathways for G protein-coupled receptors, AT2R and MasR, which are weakly defined, may interact (64). Another study also showed absence of marked RBF response to Ang 1-7 infusion in male rats (65). Consistent with our findings, it has been explained that intrarenal infusion of AT1R blockers or ACE in rates produces no changes in plasma aldosterone concentration and has little effects on systemic hemodynamics, and leads to increased sodium excretion (66-68).

In the presence of MasR while AT1R and AT2R were blocked, pressure natriuresis and diuresis increased in male rats. Such observations were not seen in female animals, which indicate the important role of MasR in pressure natriuresis and diuresis in male when both AT1R and AT2R are blocked.

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