Original Article

Solitary Functioning Kidneys Attenuate Renal Hemodynamics Responses to Angiotensin II in Male But Not in Female Rats

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Abstract

Backgrounds: People with solitary functioning kidneys (SFK) are prone to renal failure with time. Accordingly, local renin angiotensin system (RAS) and renal functions in subjects with SFK may act differently compared to normal condition. This study was designed to determine the renal hemodynamics responses to angiotensin II (Ang. II) in SFK male and female rats. **Methods:** Fifty to sixty-day-old male and female Wistar rats were subjected to unilateral renal artery obstruction, and 28 days later basal renal hemodynamic responses to Ang. II were examined in SFK groups compared to sham groups. **Results:** The findings indicated lower renal vascular resistance (RVR) and renal blood flow (RBF) responses to Ang. II in male SFK compared to sham group. Such observation was not seen in female animals. **Conclusions:** An increase in renal metabolism due to hyperfunction, especially in SFK male rats, may cause a decrease in RVR. Moreover, the lower RBF response to Ang. II may be related to alteration to Ang. II receptors in the remnant kidneys in SFK rats.

Keywords: *Animal model, renin angiotensin system, sex, solitary functioning kidneys*

Introduction

Solitary functioning kidney (SFK) is a condition associated with a reduced renal mass, atrophic kidney or loss of the kidney due to disease, damage, or removal of a kidney by surgical procedures such as trauma, cancer, donating a kidney, post-biopsy kidney hemorrhage, etc.[1-3] Also, sometimes, people are born with an un-functional kidney,[4] and there is a higher prevalence of renal damage among patients with SFK.[5] Unilateral nephrectomy at first, reduces glomerular filtration rate (GFR), but by the lapse of time, GFR tended to improve gradually. The risk of proteinuria and renal failure increase in patients with SFK,[6] and it seems that hyper-filtration in the remaining kidney can cause the onset of injury.[7] Some pathways occur in the remnants of kidney during physiological adaptation, which have negative impacts causing progression of nephron failure, including compensatory mechanisms, hypertrophy, and hyperfunction.[7,8]

The renin-angiotensin system (RAS) is a potent modulator that plays a significant role in the regulation of renal fluid homeostasis, renal hemodynamic, and vascular ton.[9] Many components of the RAS are present locally in the kidneys. In addition, there is gender difference in RAS distribution and function.[10,11] The distribution of intra-renal RAS components changes in pathological conditions or under specific situations.[12] Alteration in the expression of RAS components eventually causes changes in renal function and hemodynamics.[13] The RAS and its components' activities may disturb the subjects with SFK,[14] and atrophy of one kidney alters the RAS components in the remaining kidney, thereby affecting the renal hemodynamic response to angiotensin II (Ang. II). Accordingly, this study was designed to compare the functional state of the kidneys in SFK group, and determine the renal hemodynamic response to Ang. II in SFK rats.

Methods

Experiments were performed in 24 age-matched Wistar rats (12 males and 12 females) following approval from Animal Ethics Committee of Isfahan Medical Sciences University (Ethical Code #: IR.mui.med.rec. 1397.325). The animals were kept in standard cages and they have free access to water and rat chow. The room temperature was 23–25°C with 12h light/12h dark cycle.

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Surgical procedures

The surgical procedure was performed in two steps.

The first step applied on 50- to 60-day-old male and female rats to fasten the right renal artery to renal atrophy induction. For this purpose, rats were anesthetized with mixture of xylaxine (10 mg/kg, i.p.) and chloral hydrate (450 mg/kg, i.p.) (Merck, Darmstadt, Germany); then the right side of the abdominal wall was shaved and disinfected using Povidone-iodine 10%. After exposing the kidney, the renal artery was isolated and completely closed using a clip (U-shaped silver clip). To make the silver clip, thin silver strips with a thickness of 0.2 mm and a wide of 1-2 mm was prepared and made into a U shape.

After pushing back the kidney to the peritoneal cavity, the surgical site was sutured, and the animals kept in a recovery cage for 24 h.

In the second step, 28 days after fastening the right renal artery, animals were anesthetized with urethane (1.7 g/kg i.p., Merck, Darmstadt, Germany). Then tracheostomy was performed for adequate ventilation. Left carotid and femoral artery were isolated and catheterized by polyethylene catheter for measurement of mean arterial pressure (MAP) and renal perfusion pressure (RPP), respectively, using two separate transducers connected to the PowerLab system (AD Instruments, Sydney, Australia). Also, polyethylene catheter was implanted in left jugular vein in order to have Ang. II infusion by a microsyringe infusion pump (New Era Pump System Inc., Farmingdale, NY, USA). For the measurement of renal blood flow (RBF), the left flank was dissected by an electrical surgical cutter and, the left renal artery was isolated. Then the ultrasonic probe with a diameter of 0.7 mm (Transonic Systems Inc., Ithaca, NY 14850 USA) was placed around the renal artery. The RBF measurement was applied by flowmeter (T402, Transonic Systems Inc., Ithaca, NY 14850 USA), and renal vascular resistance (RVR) was calculated by RPP/RBF ratio. An adjustable occluder (a U-shaped metal ring with an inner diameter of 2.5 mm, in which the aorta is placed, and its inner space is adjusted by a metal rod with a diameter of 2 mm to change the diameter of aorta), also was placed around the aorta just above the renal artery (between the renal and the mesenteric arteries) to regulate RPP at normal value during Ang. II infusion.

Measurements

After the surgical procedure was completed, MAP, RPP, RBF, and RVR were recorded for 30 min continuously to reach the steady-state condition. In the last 3 min, the data were recorded as baseline data. Then Ang. II (Sigma, St Louis, MI, USA) was injected in four doses (0, 100, 300, or 1000 ng/kg/min), and each dose was administrated for a period of 15 min. At the end of each 15 min and after achieving stable conditions, data were recorded for 3 min, and the average data were reported. To control RPP at a constant level against Ang. II-induced hypertension, the diameter of the aorta was adjusted by an aortic occluder just above the renal artery. Finally, the animals were sacrificed with anesthetized drug (urethane) overdose, and the right and left kidneys were removed and weighed immediately.

Experimental design

This study was designed in four groups of experiments

Groups 1 and 2: male (n = 5) and female (n = 7) rats without atrophic kidneys (named male sham and female sham).

Groups 3 and 4: male (n = 7) and female (n = 7) rats with right atrophic kidneys (named male SFK and female SFK).

Statistical analysis

Data were evaluated using the version 20 of SPSS software (IBM, Armonk, NY, USA) and reported as mean ± standard error of mean (SEM). The baseline data in each sex were compared using the Student's *t*-test. The responses to Ang. II are presented as percentage (%) change from the values prior to administration of the Ang II. Analysis of variance (ANOVA) for repeated measures was applied to analyze the responses to Ang II.

Results

Baseline data

The baseline data for MAP, RPP, and RBF were collected after equilibrium (before Ang. II administration). No significant differences were detected for MAP and RPP in male or female between sham, and SFK groups. There were significant and insignificant differences in RBF ($P < 0.05$) and RVR between male rats in sham and SFK groups, respectively, while no significant difference detected in RBF and RVR between female groups [Table 1]. The left and right kidneys were normalized to 100 g body weight, and comparing the weight of the atrophic kidney (right kidney) in male and female SFK groups and the right kidney in sham groups indicated a significant ($P < 0.05$) decrease in the weight of the atrophic kidney, which confirms the induction of the SFK model [Table 1]. The weight of the contralateral kidney (left kidney) in male and female SFK groups was significantly higher compared to the sham group ($P < 0.05$).

Hemodynamics responses to Ang. II administration

Ang. II infusion led to dose-related increases in MAP (P dose < 0. 001 in male and P dose <0. 01 in female) in all the experimental groups [Figure 1]. There was no significant difference in MAP between sham and SFK groups in both sexes. As mentioned before, RPP was controlled by an occluder, and, therefore, Ang. II had no effect on RPP [Figure 1]. However, there was a significant difference (P group < 0.01) in RPP between sham and SKF male group which was considered unimportant.

Table 1: The baseline data for mean arterial Pressure (MAP), renal perfusion pressure (RPP), left renal blood flow per gram, left kidney weight (RBF/g tissue), left renal vascular resistance per gram left kidney weight (RVR/g tissue), left and right kidneys weights per 100-gram body weight (LKW/100 g BW and RKW/100 g BW) in male and female rats. The right kidney was subjected to necrosis in male and female SFK groups. The *P* values were obtained by the *t*-Student test

Model/ Sex	MAP (mmHg)		RPP (mmHg)		RBF (ml/min/g)		RVR (mmHg/ml/ min/g)		LKW (g/100gBW)		RKW (g/100gBW)	
	M	F	M	F	M	F	M	F	M	F	M	F
Sham	83.0±9.1	87.8±4.0	73.1±8.3	75.1±5.6	2.36±0.2	2.92±0.2	31.2±2.6	26.8±3.6	0.36±0.01	0.37±0.02	0.37±0.02	0.36±0.02
SFK	88.0±4.9	85.5±5.3	78.8±5.5	74.4±5.2	3.17±0.3	2.70±0.2	25.3±1.2	29.3±4.0	0.49±0.01	0.48±0.03	0.12±0.01	0.13±0.01
P	0.64	0.73	0.58	0.94	0.046	0.50	0.059	0.64	<0.0001	<0.01	<0.0001	<0.0001

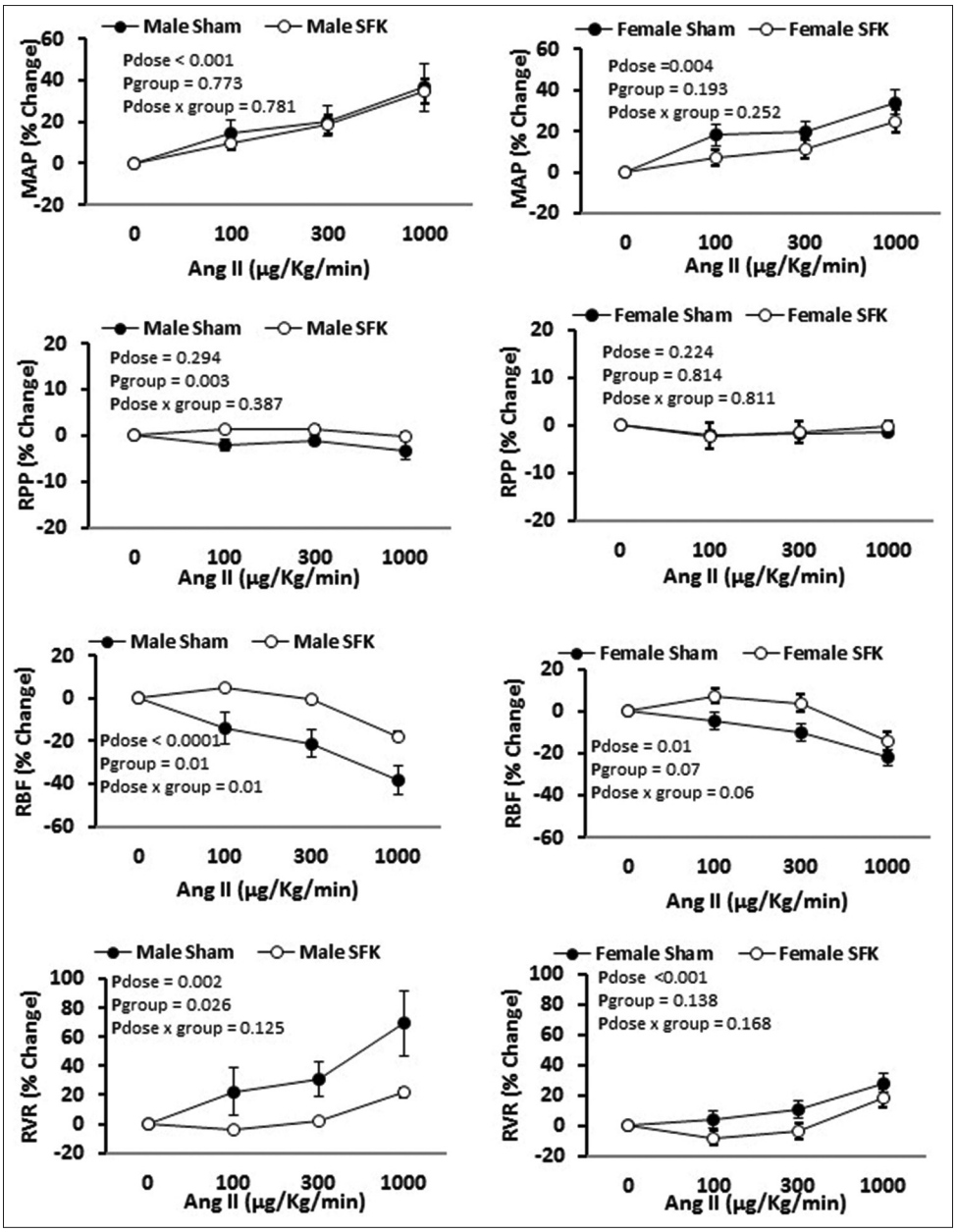

Figure 1: The effect of graded angiotensin II administration on the percentage change of mean arterial pressure (MAP), renal perfusion pressure (RPP), renal blood flow (RBF), and renal vascular resistance (RVR) in male and female rats. The right kidney was subjected to necrosis in male and female SFK groups. The *P* values were obtained by ANOVA for repeated measured data

There were lower RBF and RVR responses to Ang. II in SFK groups when compared to sham groups. However, there was a significant difference in RBF and RVR responses to Ang. II between sham and SFK

groups in male significantly (RBF: P_{group} = 0.01, RVR: P_{group} = 0.026).

Discussion

The main objective of this study was to determine the RBF and RVR responses to Ang. II administration in SFK male and female rats compared with sham animals. In the baseline data (before Ang. II infusion), RVR was insignificantly lower, and RBF was significantly higher in male rats with SFK compared with sham group, and such results were not observed in females. Also, there were significant different in RBF and RVR responses to Ang. II in male rats.

By removing one kidney, compensatory mechanisms are activated in the remaining kidney, which can affect the kidney function.[8,15] According to this study, kidney weight in remnant kidney was more than the equivalent kidneys in healthy animals. This alteration is related to hyperfiltration, hypertrophy, and hyperfunction which occur in remnant kidney during physiological adaptation.[5,7,8] It is indicated that mechanical tension due to hyperfiltration induces hypertrophic signals,[16] and RAS is one of the major mediators of hypertrophic response that can regulate fibrogenic cytokines production, reactive cell hypertrophy, and hemodynamics.[8,17,18] Moreover the induction of inflammation and cellular dedifferentiation due to the entry of large amounts of biologically active molecules into the remnant kidney, nephron and tubulointerstitium, and the molecular response to maintain homeostasis, initiates a program that eventually ends in kidney damage.[8,19]

Although an increase in kidney weight was observed in both sexes, increased RBF and decreased RVR were observed only in males. Male gender is a risk factor for cardiovascular and renal abnormality.[19] Male patients with congenital anomalies of the kidney are younger at the start of renal replacement therapy than female patients.[20] Differences in systemic or renal hemodynamics, due to the impact of sex hormones on some regulatory pathways, such as the nitric oxide production and RAS activity, may explain sex-related differences in renal complication.[21-23] The protective role of estrogen in women has been demonstrated against the negative effects of testosterone in men on kidney function.[24,25]

Some studies indicated no correlation between gender and risk for renal failure in SFK patients (especially in children).[21,22,26] In contrast, other studies showed that male SFK patients had a higher risk of chronic kidney diseases compared to female patients.[3,23] In addition, it should be mentioned here that male patients' need for more or sooner kidney transplantation might be due to many other causes besides gender like addiction, noncompliance, use or abuse of drugs, higher risk of high blood pressure, etc., which were not considered in this study.

Hyperfunction in the remnant kidney during nephron loss causes the enhancement of oxygen and ATP consumption, hypoxia, and induced acidosis and reactive oxygen species production.[8] Metabolism changes in the kidney and accumulation of metabolic substrate can affect the RVR and blood pressure.[24,25] Inadequacy of the local vascular supply in these conditions, causes hypoxia/ischemia and increases reactive oxygen species generation.[8] In general, accumulation of metabolic substrate and insufficient oxygen delivery to renal tissue may cause reducing RVR and thus increase RBF,[27,28] and it must be considered that the metabolic activity in the kidneys of males is more than females.[29]

The result of this study also indicated the lower RBF and RVR responses to Ang. II in male (significantly) and female (insignificantly) SFK rats. The reason may be related to RAS components alteration. One study explained the levels of angiotensinogen, renin and Ang. II type 1 receptor (AT1R) mRNA, and the glomerular of Ang. II receptor density after unilateral nephrectomy.[30] Another study showed lower renal cortical AT1R expression and greater AT2R/AT1R ratio in the remnant kidney in unilateral nephrectomy group compared to the sham group.[14] Therefore, possibly the lower RBF response to Ang. II in the SFK groups was related to the decreased expression of AT1R.

Conclusion

In conclusion, despite the higher basal metabolism in male, the increase in renal metabolism due to hyperfunction, and accumulation of metabolic substrate, especially in the male may be caused by a decrease in RVR in SFK male sex. Moreover, lower RBF response to Ang. II may be related to enhancement in cortical AT2R/AT1R ratio in the remnant kidneys in male SFK rats due to physiological adaptation.

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Conflicts of interest

There are no conflicts of interest.

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