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Angiotensin 1-7 administration alters baroreflex sensitivity and renal function in sympathectomized rats

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ABSTRACT

Background: Renin-angiotensin system (RAS) plays an important role in cardiovascular and kidney functions to regulate the systematic blood pressure. The baroreflex sensitivity (BRS) index is a quantitative index that was considered as a prognostic indicator for cardiovascular system risk which is known as heart rate change to blood pressure change ratio ($\frac{\Delta HR}{\Delta MAP}$). Sympathetic nerve is an arm of the BRS which may be influenced by RAS vasodilatory arm function.

Objectives: To determine the role of angiotensin 1-7 (Ang1-7) accompanied by bilateral renal denervation (RDN) on BRS and renal function.

Methods: Male and female anesthetized Wistar rats were subjected to RDN and treated with Ang1-7 (300 and 1000 $ng.kg^{-1}.min^{-1}$). Mean arterial pressure (MAP) and heart rate (HR) responses to phenylephrine (PE) infusion were measured to determine BRS index. As kidney function markers, the creatinine clearance (CrCl) and urine flow (UF) were also determined.

Results: The results showed that BRS increased significantly in RDN male rats treated with Ang1-7 compared to vehicle ($P<0.05$). BRS attenuates significantly in RDN male rats treated with vehicle or Ang1-7 when compared with non-RDN (control). In female rats, the BRS decreased significantly in Ang1-7 treated group ($P<0.05$). Also, the BRS was significantly different between non-RDN and RDN female rats treated with Ang1-7 ($P<0.05$). The ClCr in female and UF in both genders increased by Ang1-7 infusion in RDN and non-RDN rats. The alteration of serum nitrite level by Ang1-7 in non-RDN and RDN groups was gender related.

Conclusions: The Ang1-7 infusion could alter the BRS index in RDN rat's gender dependently. The ClCr response to Ang1-7 infusion in male rats was dose related.

Implication for health policy/practice/research/medical education:
To find the role of renal sympathectomy and Ang1-7 infusion in renal function and BRS index.

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1. Background

One of the main arms of renin-angiotensin system (RAS) that act against angiotensin II (AngII) is angiotensin 1-7 (Ang1-7) (1). One of the homeostatic factors that interact with RAS is baroreceptor reflex sensitivity (BRS) index. BRS in known as heart rate change to blood pressure change ratio ($\frac{\Delta HR}{\Delta MAP}$) which represents the level of baroreflex activity, vascular tone and vascular resistance (2). The BRS as a prognostic indicator for cardiovascular system risk could be altered by angiotensin II (AngII) infusion in the brain (3). However the results would be different if AngII infused systematically. Ang1-7 is one of the vasodilatory arm of RAS that not able to cross the blood-brain barrier, and it seems that the role peripheral Ang1-7 on the BRS index and renal function needs to be studied. In addition, the Ang1-7 receptor is reported to be acted gender related (4). Therefore we hypothesized that Ang1-7 infusion alters the BRS index and renal function, and this hypothesis was tested in bilateral renal denervation (RDN) and non-RDN male

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and female animal's model.

2. Objectives

To determine the role of Ang1-7 infusion accompanied by bilateral RDN on BRS and renal function in rats.

3. Materials and Methods

3.1. Animals

Male and female Wistar rats (216.3±10.2 g, n=36 & 193.7±8.97 g, n=36) were used. The animals were divided into 12 groups (n=6 in each group).

Groups 1&2: Non-RDN groups of male and female rats that were treated with saline, then received phenylephrine (PE).

Groups 3 & 4: Non-RDN groups of male and female rats that were treated with Ang1-7 (300 $ng.kg^{-1}.min^{-1}$), then received PE.

Groups 5&6: Non-RDN groups of male and female rats that received the same regimen of groups 3&4 except Ang1-7 of 1000 $ng.kg^{-1}.min^{-1}$ instead Ang1-7 of 300 $ng.kg^{-1}.min^{-1}$.

Groups 7-12: Male and female rats were subject to RDN, then received the same regimen of groups 1-6 respectively.

3.2. Experimental Surgery

3.2. 1. Surgical preparation

The rats were anesthetized with urethane (1.7 $g.kg^{-1}$ body weight, Sigma St. Louis USA), and the trachea was cannulated. In order to measure mean arterial pressure (MAP) and heart rate (HR), the left carotid artery was cannulated linked to a transducer cable to Quad Bridge Amp (ADINSTRUMENTS, model: ML224, S/N: 224-0265, Australia) connected to PowerLab 4/30 hardware (ADINSTRUMENTS, model: ML866, S/N: 430-0658, Australia) and LabChart software (ADINSTRUMENTS, v7.3.7, Australia). The RDN was performed by sectioning the left and right renal sympathetic nerves. The renal sympathetic nerves were similarly exposed and manipulated without RDN in non-RDN groups. The rectal temperature was controlled at 37± 2°C, and the bladder was cannulated for urine collection.

3.3. Experimental Protocol

3.3. 1. Baseline measurement and responses to angiotensin II

(a) Baseline; after 30 minutes of equilibration, the baseline data (the last 5 minutes) for MAP and HR were determined.

(b) Treat with Ang1-7; after equilibrium time, saline or Ang1-7 (300 or 1000 $ng.kg^{-1}.min^{-1}$) were administrated via jugular vein using micro-syringe pump (New Era Pump System Inc. Farmingdale, NY, USA). Fifteen minutes post saline or Ang1-7 infusion, MAP and HR responses to saline or Ang1-7 infusion were measured over the last 5 minutes of infusion

(c) Response to PE data; the single bolus dose of α-adrenergic receptor agonist, PE (0.1 $mg.kg^{-1}$ body weight) (5) was injected intravenously via the jugular vein. MAP and HR changes (ΔMAP and ΔHR) by the peak amplitude of pressure and bradycardia responses were measured. At the end of PE infusion, the collected urine volume and blood sample were obtained. The levels of serum and urine Cr using quantitative diagnostic kits (Pars Azmoon, Iran) and the serum level of nitrite by the Griess method were measured. The Cr clearance (ClCr) was calculated by clearance formula as follows: ClCr=Urine flow(UF)*Urine Cr level/ Serum Cr level.

3.4. Ethical issues

The experimental protocol was approved by Isfahan University of Medical Sciences Ethics Committee (Code # ir.mui.rec.1395.3.811). Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Isfahan University of Medical Sciences.

3.5. Statistical analysis

The data is reported as the mean ± SEM. Repeated measures ANOVA for MAP and HR and one-way ANOVA for ClCr, UF and serum nitrite levels were employed to compare between the groups using Tukey test as post hoc test. The Student *t* test was applied to find the statistical difference between the genders and $P \leq 0.05$ was considered to be significant.

4. Results

4.1. MAP, HR and BRS index measurements

The data for MAP and HR in RDN and non-RDN groups are shown in Figure 1. The results indicated that BRS increased significantly in RDN male rats treated with Ang1-7 (1000 $ng.kg^{-1}.min^{-1}$) compared to vehicle or Ang1-7 (300 $ng.kg^{-1}.min^{-1}$, $P<0.05$, Table 1). Moreover, BRS increased significantly in non-RDN male rats treated with vehicle or Ang1-7 when compared with RDN male rats treated in a similar manner ($P<0.0001$, Table 1). In female rats, the BRS decreased significantly in Ang1-7 (300 or 1000 $ng.kg^{-1}.min^{-1}$) compared to vehicle treated ($P<0.05$, Table 1). Also, the BRS was significantly different between non-RDN and RDN female rats treated with Ang1-7 (300/1000 $ng.kg^{-1}.min^{-1}$, $P<0.0001$, Table 1). There is also gender difference response in BRS index.

4.2. Serum nitrite, UF and ClCr measurements

The ClCr increased significantly by Ang1-7 (300 $ng.kg^{-1}.min^{-1}$) infusion in non-RDN and RDN male and female

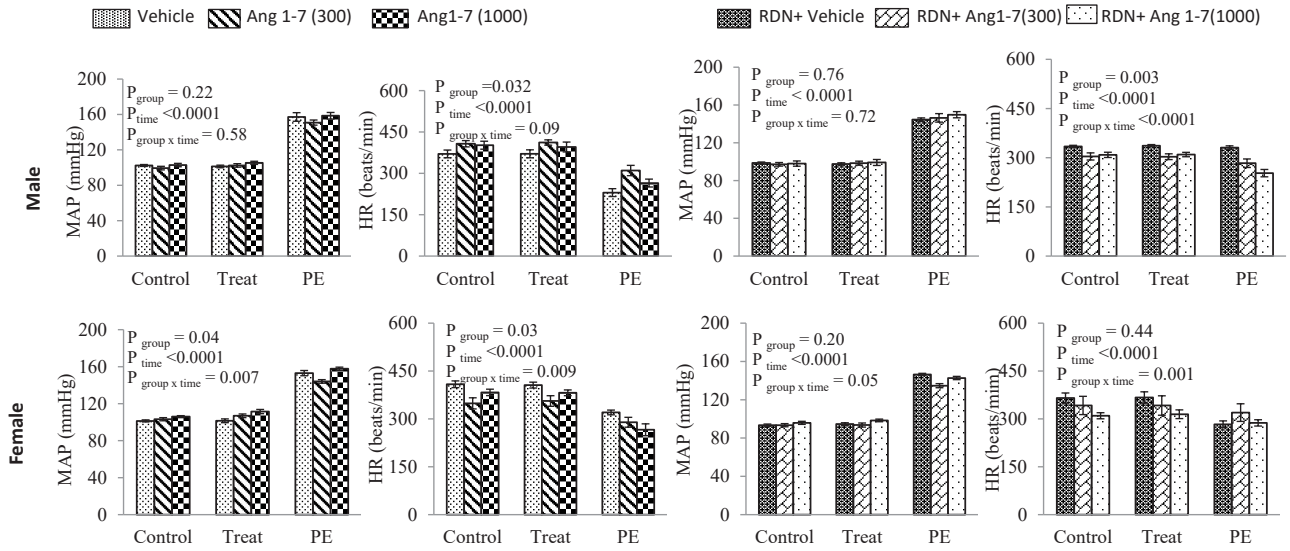

Figure 1. Data for mean arterial pressure (MAP) and heart rate (HR) in intact (non-RDN) and renal denervation (RDN) rats in equilibrium state (Control), vehicle/Ang1-7 (300/1000 ng.kg^{-1}.min^{-1}) infusion (Treat), and phenylephrine infusion (PE). Repeated measures ANOVA was applied to compare physiological parameters using factor group treatment (P_{group}), time (P_{time}) and the interaction between group treatment and time ($P_{group\ x\ time}$).

rats ($P<0.05$), however in female rats ClCr was increased significantly by high dose of Ang1-7 infusion ($P<0.05$, Table 1). Also, UF increased significantly by Ang1-7 infusion in RDN and non-RDN male and female rats ($P<0.05$). Moreover, the serum nitrite levels were significantly different between the gender in non-RDN and RDN treated with Ang1-7 (300 and 1000 ng.kg^{-1}.min^{-1}, $P<0.001$).

5. Discussion

It was reported that renal sympathetic nerves and RAS have reciprocal interaction (6). RAS components alter the renal sympathetic nerve activity (RSNA) (7). It was reported that RSNA can be altered by intravertebral injection of AngII (8). Also, it has been shown that infusion of AngII in the brain alters the baroreflex activity and RNSA (9). RDN resets or restores the baroreflex control of RSNA and reduces blood pressure in resistant hypertension (10,11). The main findings of our study indicated that the BRS increased in RDN male rats treated with Ang1-7 compared to vehicle or Ang1-7. Also, the BRS increased in non-RDN male rats compared to RDN when treated with Ang1-7 in a similar manner. Different results of BRS in different gender may be related to sex hormone as it has been shown that estrogens can modulate the BRS in normotensive rats (12,13). Also, it is possible that a lesser sympathetic output to the kidney of females may contribute to their lower blood pressure (14). RDN reduces hypertension in male SHR rats (15,16). Chen and DiCarlo reported that the performance and efficiency of baroreflex function in female were more than male rats (17). In line with our result, it is possible that elevated nitric oxide (NO) signaling increases after radio-frequency RDN and plays a role in the reduction in MAP (18). Finally, the effect of Ang1-7 on ClCr and UF seems to be related to the

Table 1. Data for baroreflex sensitivity (BRS) index, creatinine clearance (ClCr), urine flow (UF) and serum nitrite level (Nitrite) in intact (non-RDN) and renal denervation (RDN) male and female rats in the groups treated with vehicle or two different doses of Ang1-7 infusion

Group/ Parameter		Non-RDN				RDN			
		BRS index (beats.min^{-1}mm Hg^{-1})	ClCr (µLmin^{-1}g^{-1})	UF (µLmin^{-1}g^{-1})	Nitrite (µmol/L)	BRS index (beats.min^{-1}mm Hg^{-1})	ClCr (µLmin^{-1}g^{-1})	UF (µLmin^{-1}g^{-1})	Nitrite (µmol/L)
Gender/Ang1-7 (ngkg^{-1}min^{-1})									
Male	0	-2.6±0.4#	181.4±58.2	2.5±0.2	4.8±0.6#	-0.13±0.05	95.3±11.8	2.5±0.3	12.7±1.7
	300	-2.2±0.4#	347.1±66.4*	3.9±0.6*	6.4±1.2#	-0.41±0.08	480.4±148.7*	4.5±0.6*	14.1±2.0
	1000	-2.5±0.1#	121.8±6.0†	4.5±0.5*	3.9±0.3#	-1.19±0.30*†	209.8±26.0†	4.2±0.4*	12.7±1.4
Female	0	-1.7±0.1	68.9±48.7	2.9±0.2	10.0±1.8	-1.57±0.43	154.4±60.2	2.9±0.2	14.5±0.6
	300	-1.9±0.1#	479.8±95.7*	4.4±0.7*	5.4±0.4*#	-0.57±0.17*	251.6±36.7*	4.8±0.6*	16.3±2.1
	1000	-2.6±0.3#	364.5±75.4*	5.7±0.4*	6.6±0.3*#	-0.60±0.21*	804.8±409.2*	4.4±0.2*	3.5±0.32*†

Significant difference from (*) Ang (0 ngkg^{-1}min^{-1}) or (†) from Ang (300 ngkg^{-1}min^{-1}) in similar gender ($P<0.05$). The symbol of # indicates significant difference from RDN in similar gender ($P<0.05$).

vasodilatory effect of Ang1-7(1).

6. Conclusions

RDN and gender may influence BRS index and renal function in response to Ang1-7 administration.

Authors' contribution

MKA conducted the experimental procedures, helped in study design, analysis and prepared the first draft of the manuscript. MN designed, supervised and analyzed the research and completed the manuscript. All authors read and signed the paper.

Conflicts of interest

The authors declare no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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