THE EFFECT OF ANGIOTENSIN II ICV INJECTION ON HR OF OVARIECTOMIZED HYPERTENSIVE RATS

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Summary

The central interaction of sex hormones and renin-angiotensin system (RAS) in regulation cardiovascular has been suggested. In the present study the effect of Angiotensin II(Ang II) i.c.v injection on heart rate(HR) of hypertensive ovariectomized(OVX) rats was investigated. 40 female Wistar rats were divided in to 4 groups (1) Sham, (2) OVX, (3) Ovariectomized-Desoxycorticostron (OVX-Doca) and (4) Sham- Desoxycorticostron (Sham-Doca). Groups 1 and 2 received tap drinking water and were injected by oil seasom (1ml/kg; s.c.). Group 3 and 4 received NaCl (1%) and KCl (0.1%) instead of tap drinking water and treated by Doca (45 mg/kg/day). After four weeks, a polyethylene catheter was inserted into the left femoral artery in anesthetized rats. Ang II (0 (Saline), 0.0005, 0.005, 0.05, 0.5, 5 μg) were infused i.c.v and HR was recorded. There was no significant difference in HR between OVX and OVX - Doca. The Heart rate in Sham-Doca group was significantly higher than OVX-Doca group (p<0.01). The animals of Sham-Doca group had significantly higher Heart rate compared to Sham group, (p<0.05). The result of present study suggest that female sex hormones have an interaction with RAS and Ang II receptors in the central nervous system to regulate cardiovascular functions.

Keywords: Heart rate, Angiotensin II,I.C.V, Ovariectomized, Rat

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Introduction

In general, cardiovascular disease is higher in men than in age-matched premenopausal women however, there is a similar between women and men after menopause(1). Sex differences in animal models of cardiovascular disease including the spontaneously hypertensive rat and Dahl salt-sensitive rat, and ischemia-reperfusion injury have also been reported (2-4). Although the mechanisms of these sex differences are unknown, the role of sex hormones in modulating the activity of several regulatory systems, including the renin-angiotensin system (RAS), has been suggested(5). Angiotensin II (Ang II) is an octapeptide involved in functions such as blood pressure regulation, water and electrolyte balance and reproductive control. There are two forms receptor for the action of Ang II. These receptors are present in the brain areas involved in the pituitary function as well as the areas which are involved in cardiovascular function regulation (6). Recently the interaction between estrogen and central or peripheral acting ANG II has been suggested (5). It was shown that central administration of 17β-estradiol decreases the presser effect of ANG II in OVX animals(7, 8). Therefore it might be suggest that female sex hormones have a role in cardiovascular regulatory effects of AngII especially, in central nervous system. The present study aimed to evaluate the effect of AngII i.c.v injection on blood pressure of ovariectomized and naïve hyperttensive female rats.

Material and methods

Animals and drugs

Female Wistar rats, 8 weeks old (200±10 g) were used. The animals were housed in 4–5 per standard cages, at room temperature (24±1 °C) on a 12 h light/dark cycle. Food and water were available ad libitum properly. Animal handling and all related procedures were approved by the Mashhad Medical University Committee on Animal Research. Angiotensin II was purchased from Sigma (USA) and dissolved in saline. Ketamine was provided by Daroo-Pakhsh Pharm. (Iran). The animal groups were 1) Sham; 2) Ovariectomy (OVX); 3) Sham-Desoxycorticostron (Sham-Doca) and 4) Ovariectomy- Desoxycorticostron (OVX-Dca). The animals in Sham-Doca and OVX-Doca received 1% NaCl and 0.1% KCl solution (9) instead of drinking water during 4 weeks and were injected 45mg/kg Doca three times a week. The animals of Sham-Doca and OVX - Doca received tap drinking water instead of salt solution and were injected season oile instead of Doca.

In the animals of OVX-Doca ovaries and 1 kidney was removed however in OVX group ovaries were only removed. In animals of both Sham and Sham-Doca groups ovaries and kidneys were remained.

Surgery

The animals were ovariectomized under ketamine anesthesia (150 mg/kg and xylazine 0/1 mg/kg i.p.)(10, 11). Anesthesia was confirmed by reduced respiratory rate and no response to gentle pinching of foot pad. Abdominal incision was made through the skin of the flank, then ovaries and ovarian fats were removed. Ovaries were isolated by ligation of the most proximal portion of the oviduct before removal. The same procedure was performed on the sham rats except that the wound was closed without removing the ovaries. After surgery, rats were given ip 300,000 units of procaine penicillin G to prevent infection. Animals were allowed 7 days to recover from surgery(12). After 4 weeks, the animals were anaesthetized with urethane (1.2 g/kg, ip)(13). To record arterial blood pressure and heart rate, a polyethylene catheter was inserted into the left femoral artery. To permit i.c.v. injection the animals placed in a stereotaxic instrument (Stolting Instruments, USA). Stainless steel, 23-gauge guide cannulas were implanted 0.6 mm above the left lateral cerebral ventricle. Sterotaxic coordinates were selected according to rat brain atlas of Paxinos and Watson (0.9mm posterior to the bregma, lateral +1.6mm lateral to the sagittal suture and 3mm from top of skull) (14, 15). The presence of cerebrospinal fluid (CSF) in the guide cannula was examined to verify the proper placement. Cannula wasc fixed with dental acrylic cement anchored by two screws placed in the skull. A stylet (26-gauge stainless steel) was placed into the guide cannula to allow the guide cannula to maintain patency (14, 15).

Experimental design

For each animal, arterial polyethylene catheter was conneted to a transducer and powerlab(AD instrument) and the stylet was removed from the i.c.v guide cannula and a 26-gauge injection needle (1mm beyond the tip of the implanted guide cannula) was inserted. The injection needle was attached to a 10-µl Hamilton syringe by a polyethylene tube. Blood pressure was recorded as a base and the rats received the following i.c.v, bolus injections of Ang II ; 0 (Salin), 0.0005, 0.005, 0.05, 0.5, 5µg and heart rate was measured(16). The volume of i.c.v. injection was 5 µl.

Histology

Immediately after the tests, all rats were given 2µl of methylene blue in a lateral ventricle, and they were anesthetized with a high dose of urethane and ranscardially perfused with 100 ml of saline followed by 100 ml of formalin (10%). The brains were removed and placed in formalin (10%). After 3 days, the brains were sliced into 60-µm-thin slices. Data from rats with incorrect placement were excluded from the analysis (10, 11, 14, 15).

Data analyses

All data were expressed as the means، SEM. The data of different groups were compared using repeated measured ANOVA tests with Tukey's post hoc analysis between groups. The criterion for statistical significance was (p<0.05).

Results

The base BP in both Sham- Doca and OVX-Doca groups was significantly higher than Sham and OVX groups respectively (p<0.001 and p<0.01) After i.c.v injection of Ang II. There was no significant difference in HR between OVX and Sham groups(fig1A). The Heart rate in Sham-Doca group was significantly higher than OVX-Doca group (p<0.01)(fig 1B). The animals of Sham-Doca group had significantly higher Heart rate compared to the animals of Sham group(p<0.05) (fignA). There was no significant difference in HR between OVX-Doca and OVX groups (fig2B).

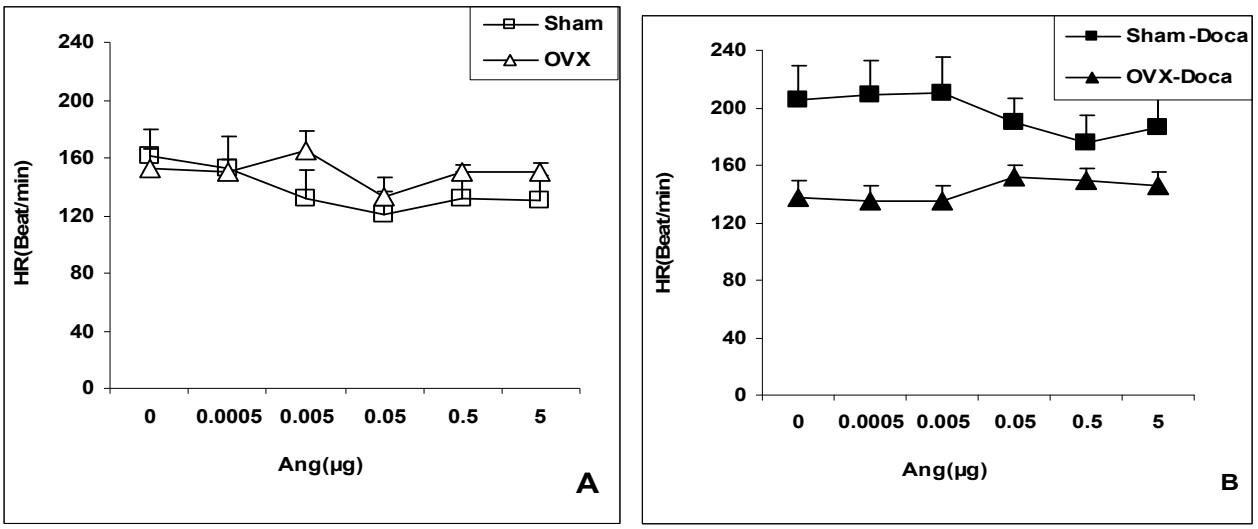

Fig 1: Comparison of Heart rate between Sham and OVX(A), Sham-Doca and OVX-Doca(B) rats following i.c.v injection of Ang II. Data were presented as mean ± SEM of beat/ minute(n=10 in each groups). There was no significant difference between Sham and OVX roups using repeated measure ANOVA test. The Heart rate in Sham-Doca group was significantly higher than OVX-Doca group (p<0.01).

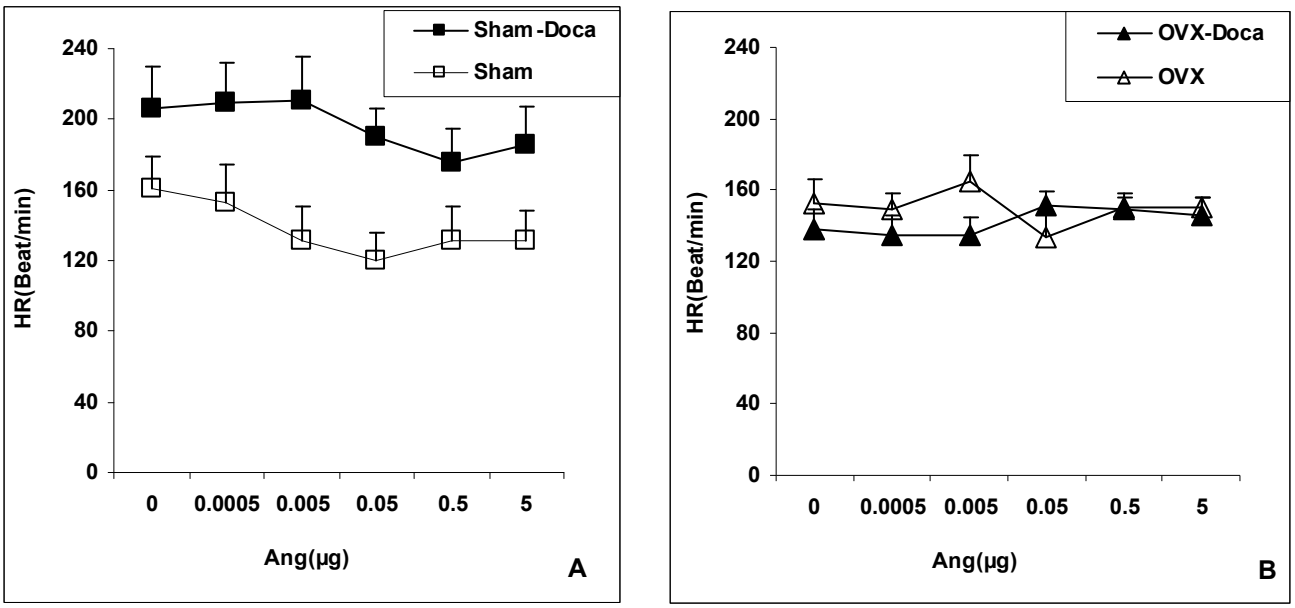

Fig 2: Comparison of Heart rate between Sham-Doca and Sham(A), OVX-Doca and OVX(B) rats following i.c.v injection of Ang II. Data were presented as mean ± SEM of beat/ minutes (n=10 in each groups). The animals of Sham-Doca group had significantly higher Heart rate compared to the animals of Sham group(p<0.05). There was no significant difference between OVX-Doca and OVX groups using repeated measure ANOVA test.

Discussion

The results of epidemiological studies imply that the incidence of cardiovascular diseases is different between women and men with similar ages. It has also shown that hormone repleacement therapy reduces the risk of postmenopausal cardiovascular complications (17-19). Thus, it has been suggested that ovarian hormones have an important role in regulation of cardiovascular functions(20). It has been recently shown that sex hormone especially estrogen may affects cardiovascular regulator centers in the brain(5). Co- operation of estrogen and angiotensin peptides in the brain has also been suggested (8). Therefore, we hypothesized that centrally acting Ang II may have an interaction with estrogens in regulation of heart rate.

Doca-Salt treated rats have been frequently used as a model of hypertension. The results of present study showed the blood pressure in both OVX- Doca and sham- Doca groups was greater than OVX and Sham groups. We examined the i.c.v injection of Ang II on HR in the presence and absence of ovarian hormones as well as in normotensive and hypertensive rats.

The result showed no significant difference in HR after i.c.v injection of repeated doses of Ang II between Sham and OVX groups. There are many studies showing the cardiovascular protective effects of estrogens(21). It has been frequently reported that the removal of ovaries, the main source of circulating estrogen in female, facilitates the development of blood pressure(22, 23). The beneficial effects of estrogen have been attributed to endothelium-dependent vasodilation and a direct effect of the hormone on endothelial cells. It has also been shown that administration of estrogen upregulates the transcription of NO synthase, the key enzyme regulating NO production(24). In addition, a further possible explanation is suggested by the finding that estrogen has antioxidant properties and may preserve endothelium-dependent vasodilation through the negative effect of superoxide anions that strongly inactivate NO(25). Estrogen may directly improve lipid profile by decreasing plasma concentrations of total and LDL cholesterol while increasing high-density lipoprotein cholesterol, and inhibiting oxidation of the LDL fraction(26). In contrast, the results of other studies showed that estrogen may have no effects or even negative effects in blood pressure or cardiovascular system(27). The results of present study also showed that deletion of ovarian hormones didn't change that the central effect of angiotensin II on HR. It seems that there is no interaction between sex hormones and angiotensin II for regulation of HR in physiological conditions(28). On the other hand the results also showed that HR in Sham-Doca group was higher than Sham groups, In contrast there was no significant difference between OVX-Doca and OVX groups in HR following i.c.v Ang II administration. It might be suggested that ovarian hormones affects the central function of RAS in regulation of cardiovascular system in hypertensive conditions(5).

The important role of brain renin-angiotensin system in sodium homeostasis and central cardiovascular regulation has been widely documented. All components of the brain RAS present in regions such as circumventricular organs, the paraventricular nucleus, median preoptic nucleus and suprachiasmatic nucleus(29). It has been reported that Doca stimulates AT1 binding in these areas and it may affect the AngII-mediated functions in this way (30)**.** It has been also shown that salt-induced hypertension in Dahl S rats is accompanied by increased densities of AT1 receptors in brain areas both inside and outside of the BBB (31).

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