Original Article

Involvement of β-adrenergic receptor of nucleus tractus solitarius in changing of baroreflex sensitivity by estrogen in female rats

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Abstract

Background: Arterial baroreflex (ABR) is an important factor in preventing of blood pressure fluctuations that determined by baroreflex sensitivity (BRS). Estrogen is an ovarian hormone that has influence on ABR. The mechanism of this effect of estrogen unknown and may be mediated by β-adrenergic receptor of nucleus tractus solitarius (NTS), an important area in regulation of baroreflex. Therefore, in this study changing of BRS by estrogen after blockade β-adrenergic receptor of NTS in ovariectomized rats (Ovx) and Ovx treated with estrogen (Est) was examined.

Materials and Methods: After ovariectomy, all female rats divided to Ovx and Ovx + Est groups and two series of experiments were performed. In the first experiment, phenylephrine was [intravenously, IV] injected in both the Ovx and Ovx + Est groups, and mean arterial pressure (MAP), heart rate (HR), and BRS were evaluated ($n = 8$ for each group). In the second experiment, each of Ovx and Ovx + Est groups divided into saline and propranolol (pro) groups, saline and pro stereotaxically were microinjected into NTS, respectively. Further, phenylephrine (IV) was injected in all groups and BRS was evaluated.

Results: BRS significantly increased in estrogen-treated groups (Ovx + Est) compared to Ovx groups ($P < 0.01$). The blockade β-adrenergic receptor of NTS by pro did not significantly changed BRS in both Ovx and Ovx + Est groups.

Conclusion: We concluded that there aren't any intraction between estrogen and β-adrenergic receptor of NTS in BRS.

Key Words: β-adrenergic receptor, Baroreflex sensitivity, Estrogen, Microinjection, Mean arterial pressure, Nucleus tractus solitarius

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INTRODUCTION

In the recent years, incidence of cardiovascular diseases especially hypertension dramatically increased in the worldwide.[1] The mechanism(s) of hypertension is not completely understood, but several factors such as baroreceptor reflex impairments are

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involved. Previous studies reported that reduction in baroreflex sensitivity (BRS) and/or resetting of the baroreflex curve toward high pressure improves the hypertension condition.[2-4]

The nucleus tractus solitarius (NTS) is the principal site for baroreceptor reflex, and its neurons are essential component of the central pathways that mediate the cardiovascular reflexes and regulate blood pressure (BP).[4-6] The NTS receives inputs from several areas of brain and contains a wide variety of neurotransmitters including noradrenaline.[7,8] Injection of noradrenaline into the NTS in anaesthetized rats caused the depressor effect and in unanesthetized rats elicited the pressor effect.[9,10]

In addition, microinjection of propranolol (pro) (a β-adrenergic antagonist) into the NTS causes a dose-dependent increase in mean arterial pressure (MAP) and a decrease in heart rate (HR) in anesthetized rats.[9]

Estrogen (Est) is an ovarian hormone that has various effects on the central nervous system (CNS) and peripheral organs of body including cardiovascular system.[11] The influence of Est on central cardiovascular circuits also is reported.[12,13]

Previous studies have been demonstrated that local injection of Est into several autonomic nuclei such as NTS reduced renal sympathetic nerve activity, sympathetic tone, and alter BRS.[12,14,15] Although several effects of Est on cardiovascular system especially BRS are reported, its mechanism effect on the central cardiovascular regulation and BRS poorly understand. However, there is evidence that Est interacts with several neurotransmitters in brain. Because both Est and adrenergic receptors are identified in NTS, interaction of β-adrenergic receptor of NTS with Est in changing of BRS was examined in female rats in this study.

MATERIALS AND METHODS

Animals

Experiments were carried out on 40 female Wistar rats (8 weeks old and weighted 240 ± 20 g). All rats were housed in the same room under a constant temperature (22 ± 2 °C) and illuminated 7:00 a.m. to 7:00 p.m., with food pellets and water available *ad libitum*.

Surgical procedure

At first, due to the removal effect of the estrous cycle on the Est level, ovaries of all rats were removed after anesthesia with ketamine (150 mg/kg; intraperitoneally (IP)). After ovariectomy (Ovx), the rats were divided into two groups: Ovx and Ovx + Est. In the Ovx + Est group, estrogen capsules (3.8 in diameter and 30 mm long with 0.07 mm volume, WPI, USA) were implanted beneath rats skin. In the Ovx group, a silastic capsule containing of corn oil also was implanted. The rats were then allowed to recover for 2 week.

Blood pressure recording

In the day of experiment for recording of BP and HR, the animals were anesthetized with urethane (Sigma, 1.4 g/kg, IP).[16] The left femoral artery was cannulated with a polyethylene tube (PE-50) filled with heparinized saline for recording BP and HR. Femoral venous was also cannulated for drug injection. The arterial catheter connected by a pressure transducer to Power lab (AD instrument, Australia) system, and BP and HR were continuously recorded.

Baroreflex testing

To determine the effect of Est on baroreceptor reflex, after 10 min recording of BP and HR a bolus of phenylephrine hydrochloride (an α-adrenergic receptor agonist; 16 μg/kg; Sigma) was injected IV in both groups. The changes in MAP and HR were calculated and then the BRS index was evaluated. This index is calculated by dividing the changes in HR to the MAP as follows.[17]

$BRS = \Delta HR/\Delta MAP$.

Microinjections

Drug microinjections into NTS were performed according to the previous studies. The rats were placed in a stereotaxic apparatus (Stoelting, USA). A hole was drilled on the skull and injections in a volume 300 nL were made via a stainless steel injection needle. The used coordinates were 1 mm caudal to the interaural line, 0.5 mm lateral to the midline, and 8.9 mm below the skull.[18] For injection pro and saline, a polyethylene tubing (PE-20) was connected to a 5-μL Hamilton syringe filled by the drug and connected to needle injection and 300 nL of drug solution microinjected slowly into NTS.

Experimental procedure

In the first set of experiments, to determine the effect of Est on baroreceptor reflex, in both OVX and Ovx+ Est groups after 10 min recording of BP and HR a bolus of phenylephrine hydrochloride (Phe; an α-adrenergic receptor agonist; 16 μg/kg; Sigma) was injected IV and changes of MAP and HR were calculated.

In the second set of experiments, each Ovx and Ovx + Est group was divided into the pro and saline groups. Then, pro and saline were separately microinjected into NTS by a stereotaxic apparatus in both the Ovx and Ovx + Est groups. The changes in MAP and HR were calculated and then BRS was evaluated.

Experimental groups

The following groups were used (n = 8 for each group):

1. Ovx
2. Ovx + Est
3. Ovx; received phenylephrine
4. Ovx + Est; received phenylephrine
5. Ovx+ pro; phenylephrine injected after microinjection of pro into NTS
6. Ovx + saline; phenylephrine injected after microinjection of saline into NTS then phenylephrine injected
7. Ovx + Est+ pro; phenylephrine injected after microinjection of pro into NTS
8. Ovx + Est+ saline; phenylephrine injected after microinjection of saline into NTS

Data analysis

Data are presented as means ± SEM. Changes in BP and HR were determined. The maximum change was compared with the control using the unpaired t-test. The criterion for the statistical significance was $P < 0.05$.

RESULTS

Baseline cardiovascular value in Ovx and Ovx + Est female rats

Baseline systolic, diastolic, MAP, and HR in the Ovx and Ovx + Est groups are shown in Table 1. The cardiovascular parameters were not significant between Ovx and Ovx + Est groups.

Effects of phenylephrine on changes of MAP and HR and baroreflex sensitivity in the Ovx and Ovx + Est groups

Injection of phenylephrine (16 μg/kg; IV) in the Est treated group significantly increased MAP and decreased HR. However, changes of MAP in the Est-treated group were significantly lower than the Ovx group (ΔMAP: Est treated: 21.7 ± 2.2 mm Hg vs. Ovx: 45.4 ± 3.18 mm Hg) t-test, $P < 0.01$, n = 8) rats [Figure 1a].

Changes in HR in the Ovx + Est group were significantly higher compared to the control group (ΔHR: Est treated: 32 ± 3 beats/min vs. control: 23.1 ± 5.2 beats/min, $P < 0.05$, n = 8) [Figure 1b].

The BRS induced by phenylephrine was also evaluated. The BRS significantly increased in the Ovx + Est group compared to the Ovx group (BRS: Ovx + Est: 1.48 ± 0.28 vs. Ovx: 0.51 ± 0.08) (paired t-test; $P < 0.01$, Figure 2).

Effects of blockade of β-adrenergic receptor in NTS on baroreflex sensitivity in the Ovx and Ovx + Est groups

To determine whether the response to Est was mediated by activation of β-adrenergic receptor, pro (a β-adrenergic receptor antagonist) and

Table 1: Basal cardiovascular parameters in the Ovx and Ovx + Est female rats

Parameters	Female rats	
	Ovx	Ovx + Est
Systolic pressure (mm Hg)	107±7.1	103±8.2
Diastolic pressure (mm Hg)	76±5.6	103±8.2
MAP (mm Hg)	83.6±6.9	103±8.2
HR (beats/min)	285±14	103±8.2

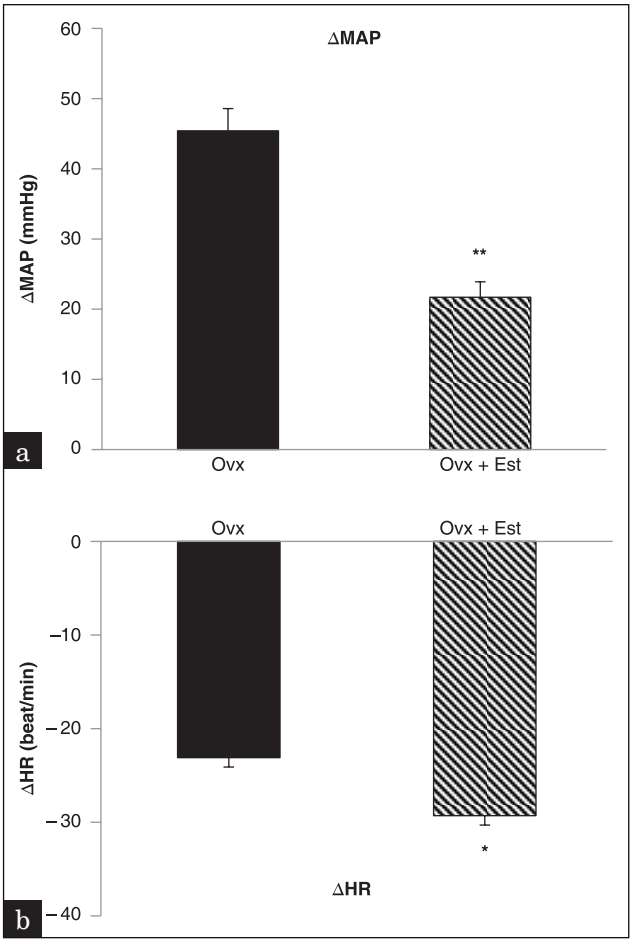

Figure 1: Changes in MAP and HR to injection of phenylephrine in Ovx and Ovx + Est female rats. Changes of MAP in the Ovx + Est group are significantly lower than the Ovx group ($P < 0.01$; n = 8, a) and changes of HR in the Ovx + Est are significantly higher than the Ovx group ($P < 0.05$; n = 8, b). *P<0.05, **$P < 0.01$

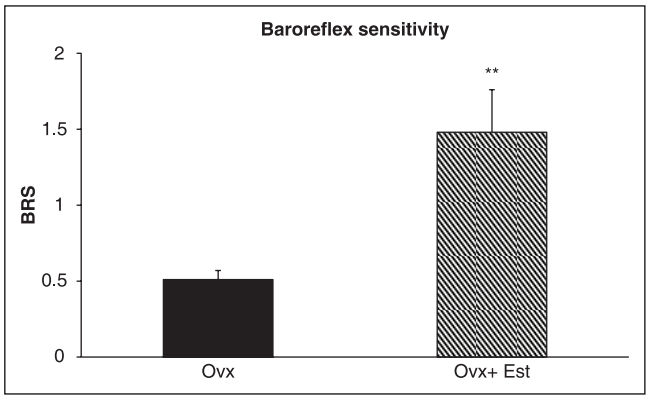

Figure 2: BRS evoked by IV injection of phenylephrine in Ovx + Est and Ovx female rats. BRS significantly increased the Ovx + Est group compared to the Ovx group ($P < 0.01$; $n = 8$). $^{**}P < 0.01$

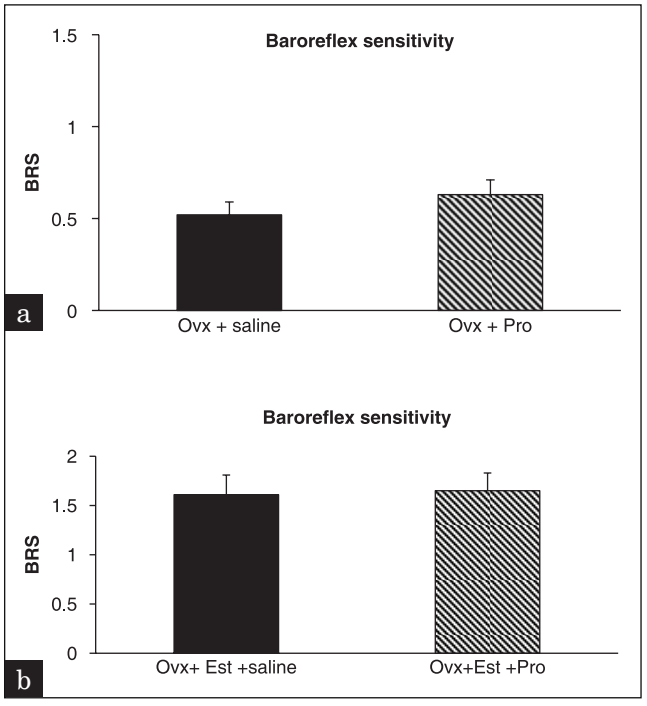

Figure 3: BRS evoked by injection of phenylephrine in the Pro and saline groups of Ovx (a) and Ovx + Est (b) rats. Microinjection of propranolol cannot significantly changed BRS compared to the saline group in both the Ovx and Ovx+ Est groups. $n = 8$

saline were separately microinjected into the NTS in both the Ovx and Ovx + Est groups. Further, baroreflex was evoked by phenylephrine (16 μg/kg, IV) injection. In Ovx animals, microinjection of pro (10/nmol, 200/nL, $n = 8$) into the NTS had not significant effect on BRS compared to microinjection of saline ($n = 8$) (BRS: Ovx + pro: 0.63 ± 0.08 vs. Ovx + saline: 0.52 ± 0.07; $P > 0.05$; Figure 3a). In the Ovx + Est group microinjection of pro into the NTS had not significant effect on BRS compared to the saline group (BRS; Ovx + Est + pro: 1.65 ± 0.18 vs. Ovx + saline: 1.61 ± 0.2; $P > 0.05$; Figure 3b).

DISCUSSION

In this study the blockade effect of β-adrenergic receptors in NTS on BRS in the Ovx and Ovx + Est groups were evaluated. Our results indicated that BRS was not changed by blockade of β-adrenergic receptors of NTS in both the Ovx and Ovx + Est groups.

BRS is an accepted clinical diagnostic test for individuals at risk of cardiovascular disease.[2,3] The baroreceptor reflex circuit is complicated and several areas are involved. One of the important brain areas is the NTS that received baroreceptors afferent and has projection to the central areas involved in cardiovascular regulation such as rostral ventrolateral medulla (RVLM).[19] The presence of Est receptors in the baroreflex pathway, especially NTS, has been shown in the previous studies.[14,19] Est has facilitator effects on baroreflex activity.[14,20] For example, Pamidimukkala *et al*. analyzed the effects of Est baroreflex responses to phenylephrine, Angiotensin II, and sodium nitroprusside and showed that this reflex facilitated by Est.[21] Consistent with this evidence, our results showed a facilitator effect of Est on BRS.

The NTS is also known contain both cell bodies and nerve terminals of the catecholaminergic system.[7,22] It is reported that the catecholaminergic system in the NTS of unanesthetized rats has important effect on processing of the sympathoexcitatory component of the baroreflex activation.[8] The β-adrenergic receptors identified in NTS and play a role in the regulation of arterial BP and HR.[10,23] Based on this evidence, we suppose that Est and β-adrenergic receptor may be interacted in baroreflex activity. Therefore, the β-adrenergic receptor of NTS is blocked by pro and then BRS is elicited by phenylephrine in Ovx and Ovx + Est. We observed that microinjection of pro into the NTS did not significantly effect on BRS in both groups. Therefore, these results did not provide interaction between the β-adrenergic receptor and Est for regulation of BRS in NTS . However, we cannot certainly rule out this interaction in baroreflex activity.

The baroreflex circuit is very complex and has reciprocal connections with many of cardiovascular regulation areas[24] and in this study we concentrated primarily on the NTS area that has a pivotal role in baroreflex activity. Therefore, maybe other areas of the baroreflex pathway that contain the β-adrenergic receptor mediate interaction of Est and β-adrenergic in modulation of the baroreflex activity. For example, Saleh *et al*. reported that the injection of Est into ambiguous and parabrachial nuclei in Ovx rats enhanced phenylephrine-induced reflex changes in

HR.[15,25,26] Therefore, it is suggested an interaction between β-adrenergic and Est in ambiguous and parabrachial areas. In present time, interaction of adrenergic and Est in modulation of baroreflex activity is not completely elucidated and need future studies.

In summary, the present experiment does not support the hypothesis interaction of Est with β-adrenergic receptor of NTS in modulation of BRS.

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