

Role of 17-beta estradiol in baroreflex sensitivity in the nucleus tractus solitarii via the autonomic system in ovariectomized rats

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ABSTRACT

الأهداف: دراسة تأثير هرمون الاستروجين المبدولة من خلال نظام الاستقلال الذاتي في سبيل النواة المفردة (NTS) على زيادة حساسية النظام العصبي في ظل ظروف ارتفاع ضغط الدم الحاد في الفئران مستأصلة المبيض.

الطريقة: أجريت دراسة تجريبية في جامعة كرمان للعلوم الطبية، كرمان، إيران خلال الفترة من مارس 2010م إلى أكتوبر 2010م، واشتملت على 36 فئران أنثى مستأصلة المبيض ومن ثم تم زرع كبسولات تحت الجلد هرمون الاستروجين. بعد أسبوعين، تم مقنى الفخذ الأيسر في الوريد والشريان عن طريق ضخ فينيليفرين وتسجيل معدل الضغط الشرياني ومعدل ضربات القلب. وبعد ذلك تم حقن الأتروبين، وبروبرانولول ومحلول ملحي في سبيل النواة المفردة، بعد ذلك تم قياس التغيرات في معدل ضربات القلب والتغيرات في الضغط الشرياني قبل تسرب فينيليفرين.

النتائج: كانت هنالك ارتفاع في هرمون الاستروجين والذي يمنع من ارتفاع معدل الضغط الشرياني أي بعد ضخ فينيليفرين، كان التغير في معدل ضربات القلب (أقل بكثير في مجموعة الاستروجين المستقبلية مقارنة مع مجموعة التحكم) ($p < 0.05$). ارتفعت حساسية النظام العصبي في مجموعة الاستروجين المستقبلية مقارنة مع مجموعة التحكم ($p < 0.01$). كما كان حساسية النظام العصبي الموهن بشكل ملموس لدى مجموعتي (الاستروجين المستقبلية والشاهد) بعد حقن الأتروبين مقارنة ببروبرانولول أو بعد حقن محلول ملحي ($p < 0.01$).

خاتمة: من المحتمل أنه في ظل ظروف ارتفاع ضغط الدم الحاد، وهرمون الاستروجين مما له تأثير على NTS من خلال نظام خاص بالجهاز السمبتاوي ويعزز حساسية النظام العصبي.

Objective: To investigate the effect of estrogen exerted through the autonomic system in the nucleus tractus solitarii (NTS) on increasing the sensitivity of the baroreflex under conditions of acute hypertension in ovariectomized rats.

Methods: In this experimental study, conducted in Kerman University of Medical Sciences, Kerman, Iran from March 2010 to October 2010, 36 female rats were ovariectomized and then estrogen capsules were implanted beneath their skin. After 2 weeks, the left femoral vein and artery were cannulated for phenylephrine infusion and recording of mean arterial pressure and heart rate. Subsequently, atropine, propranolol, and saline were injected into the NTS, followed by measurements of changes in heart rate and changes in mean arterial pressure just prior to phenylephrine infusion.

Results: Estrogen increased the bradycardia response and inhibited the rise of mean arterial pressure; namely, after phenylephrine infusion, the change in heart rate was significantly lower in the estrogen-receiving group compared with the control group ($p < 0.05$). Baroreflex sensitivity was significantly increased in the estrogen-receiving group compared with the control group ($p < 0.01$). Baroreflex sensitivity was significantly attenuated in both groups (estrogen-receiving and control) after atropine injection, compared with after propranolol or saline injection ($p < 0.01$).

Conclusion: It is probable that under conditions of acute hypertension, estrogen affects the NTS through the parasympathetic system and enhances baroreflex sensitivity.

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Hypertension is among the most serious challenges of modern societies. It is a common and asymptomatic disease, which is easy to diagnose and treat; however, if untreated, it results in fatal complications.¹ The prevalence of hypertension in women is closely related to age,² increasing considerably after the age of 50 through an unknown mechanism.¹ An appropriate systemic arterial pressure is the only requirement for optimal cardiovascular function.³ Arterial pressure is persistently monitored³ by different receptors throughout the body.⁴ The baroreceptor reflex is among the short-term regulators of arterial pressure.⁴ This reflex starts at the site of tension receptors (called baroreceptors) in the wall of systemic arteries. The afferent fibers terminate mostly at the nucleus tractus solitarius (NTS).^{5,6} According to the previous studies, the fatality of cardiovascular disease is greater in menopausal women and men compared with women before menopause.⁷⁻¹⁰ The NTS is at the main terminal point for visceral afferent fibers of the brainstem and plays an essential role in regulating cardiovascular function.^{6,11-14} A study by Saleh and Connell et al¹⁵ indicated that direct infusion of estrogen in the central nuclei of male rats caused significant changes in autonomic and cardiovascular parameters.¹⁵

Sexual differences influence the neuro-humoral control of the cardiovascular system with women having a more prominent vagal tone and thus less fatality compared with men.¹¹ Estrogen enhances the baroreflex sensitivity (BRS) under acute conditions and inhibits dramatic changes of the mean arterial pressure (MAP), thus preventing its abrupt increase, as well as intensifying the bradycardia reflex caused by phenylephrine (PE) in rats.¹⁶ Estrogen infusion in the central nuclei controlling the cardiovascular system contributes to processing and summarizing of autonomic responses.¹⁵ Estrogen increases the BRS considerably and blocks the attenuated BRS following vagal stimulation.¹⁶ It also decreases the neural stimulation in the parabrachial nucleus (PBN) through modifying the synaptic transmission via increasing the release of gamma aminobutyric acid (GABA) and decreasing the release of glutamate.¹⁷ This substance enhances vagal activity and attenuates renal nerve activity resulting in enhanced baroreflex

sensitivity and modifies the base autonomic tone via the activity of central estrogen receptors.¹⁸ Our experiments indicate that estrogen affects the BRS mostly through the parasympathetic component of the autonomic system in the NTS. The results of previous studies indicate that the autonomic inductions of estrogen on the PBN affect the glutamate receptors to decrease heart rate (HR) and increase the parasympathetic tone, while it reduces blood pressure and sympathetic activity by affecting the GABA receptors.¹⁹

Considering these facts and the prominent role of the autonomic system in regulating the cardiovascular system, as well as the role of the NTS in this regard, it is probable that estrogen enhances BRS through the autonomic system in the NTS under conditions of acute hypertension. Previous studies have proven the role of the NTS in controlling acute hypertension in animals.²⁰ Nervous stimulations from baroreceptors of the aorta and carotid arrive at the NTS and from there, the inhibitory effects are dispatched to various parts of the brainstem, resulting in decreased sympathetic flow and thus reduced blood pressure.²¹ However, damage of the NTS reduces baroreflex control of the blood pressure, leading to increased sympathetic activity and severe hypertension.²² Arterial pressure is controlled by the autonomic nervous system and the impulses generated in visceral receptors are transmitted to the CNS via the autonomic afferent pathway to be summarized in different levels and then transmitted to the operating organs via the efferent pathways.⁵ One issue that has been investigated is the role of sex hormones in controlling blood pressure.²³ Previous studies have indicated that hormones secreted by the gonads contribute to the control of blood pressure in rats.²¹ This finding resulted in introduction of sex hormone replacement therapy,^{22,23} which entailed controversial opinions.²⁴ A series of experiments indicated that ovariectomy renders the animal subject to hypertension, which was not related to the sodium content of its diet.²⁵⁻²⁷ According to the research data, estrogen is synthesized and released in the brain,^{28,29} and there are estrogen receptors in the central autonomic nuclei.³⁰

Measurements of BRS indicates BRS to be greater in women before menopause compared with men of the same age and menopausal women.^{16,31,32,33} The central mechanisms of these effects, however, remain to be discovered. In our previous study, we indicated that estrogen enhances the BRS via the autonomic system in male rats.³⁴ The objective of the present study was to investigate the mechanism of estrogen effect on autonomic activity of the NTS in female rats following ovariectomy, in order to provide a basis for

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understanding the protective cardiovascular effects of estrogen and its central mechanisms.

Methods. This is experimental study was conducted in Kerman University of Medical Sciences, Kerman, Iran from March 2010 to October 2010. The study protocol was approved by Research Deputy at Kerman University of Medical Sciences, Kerman, Iran.

We used 6 groups of animals, each with 6 rats, yielding a total of 36 laboratory rats. Our animals were 2 month old albino Wistar rats with an average weight of 250 ± 50 g. All rats were preserved under standard laboratory conditions in the vivarium of Rafsanjan University of Medical Sciences. After ovariectomy, the rats were divided into 2 groups: one receiving estrogen (18 rats) and one receiving sesame oil (18 rats, the control group). Subsequently, each group was subdivided into 3 groups for microinjection of each of the 3 drugs: atropine, propranolol, and saline into the NTS. The experiment was performed in the following stages:

Anesthesia. The rats were put under anesthesia in 2 stages. The first stage was for implanting the capsules and ovariectomy, performed with intra-peritoneal injection of 150 mg/kg of ketamine, causing transient anesthesia. In the second stage, performed 2 weeks later for cannulation in order to provide access to the NTS, 150 mg of urethane per 100 g body weight was injected intra-peritoneally and an extra dose (10 mg per 100 g body weight) was used, if required.²¹

Capsule implant. A silastic capsule 3.8 mm in diameter, 30 mm in length, and 0.07 mL in volume was implanted subcutaneously. A small incision was made on the nape and the capsule containing estrogen or sesame oil (as dissolvent), was planted under the skin. Thus, the animals fell into either of the 2 groups: receiving estrogen or receiving sesame oil.¹⁵

Ovariectomy. Two weeks prior to cannulation, bilateral incisions of 2-3 centimeters were made on the flanks of the rats to dissect the skin and muscles, revealing the ovaries. Subsequently, the ovaries were dissected and muscles and skin were repaired separately.²⁶

Cannulation. In order to facilitate respiration during anesthesia, a PE240 cannula was inserted in the trachea. The left femoral artery and vein were cannulated using PE50 cannulae containing saline and heparin (200 u/mL). The venous and arterial cannulae were used for drug infusion and pressure recording. Therefore, the arterial cannula was attached to a pressure transducer.²¹

Access to the NTS for microinjection of drugs. After cannulation, the animals were transferred to a stereotaxic instrument (Stoelting, Wood Dale, IL,

USA). The posterior surface of the medulla was exposed by craniotomy. The landmarks of the NTS were traced using a Paxinos Atlas. The scriptorius was considered as the reference point and microinjection of the drugs was performed at this point.²³

Microinjection of drugs in the NTS. Three groups were treated with 3 microinjections into the NTS: 1) atropine as a muscarinic inhibitor, 2) propranolol as a β -blocker, and 3) saline as placebo. Drugs were injected by pressure.

Recording pressure and heart rate. The arterial cannula containing saline and heparin was attached to a pressure transducer to record pressure. The pressure curve, consisting of systolic and diastolic pressures, was recorded and the data generated by a tachograph and an analyzer was processed with Power lab and HRs and MAP were calculated.

Investigating baroreflex sensitivity. For this purpose, PE was used as a vessel constrictor to cause abrupt hypertension. Following administration of PE, the ratio of the peak changes in the magnitude of the reflex bradycardia (the change in HR or Δ HR) to the magnitude of the PE-induced pressor response (the change in MAP or Δ MAP) was calculated as an index for BRS.¹⁵

Controlling the sites of microinjection with histological sections (exclusion criterion). At the end of the experiment, the brains were extracted, fixated by 10% formalin, and frozen sections of the NTS were prepared with thicknesses of 40 μ m. The slides were compared for the landmarks depicted in the Paxinos Atlas, and in case of incompatibility, they were discarded and their data were excluded from the analysis (Figure 1).

Statistical analysis. To compare BRS before and after intervention, the paired t-test was used. The means of groups were compared using analysis of variance (ANOVA) and Tukey's test was used as a post-hoc test. The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 15.00 for Windows was used for data analysis, and *p*-values equal or less than 0.05 were considered significant.

Results. As Table 1 depicts, there is no statistically significant difference between the systolic and diastolic blood pressure and the MAP of the estrogen-receiving group and the control group. In other words, both groups of rats were hemodynamically normal and identical in terms of systolic and diastolic blood pressure and MAP prior to the condition of acute hypertension. Furthermore, as the table illustrates, there was no statistically significant difference between the HR of

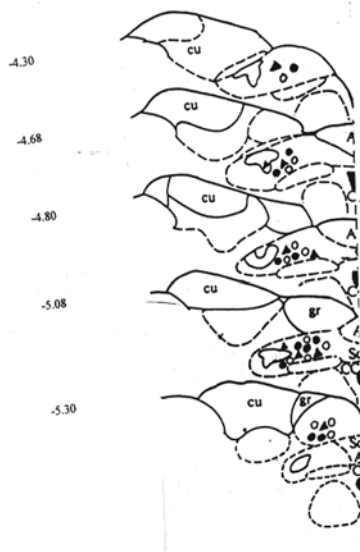


Figure 1 - Presentation of the histological sections from the nucleus tractus solitarii of ovariectomized rats where the solid circles represent the site of atropine microinjection, solid triangles represent the site of propranolol microinjection, and hollow circles represent the site of saline microinjection. The negative numbers on the left depict the distance of the incision from the interaural line according to the Paxinos Atlas. cu - nucleus cuneatus, gr - nucleus gracilis

Table 1 - Comparison of the mean systolic and diastolic blood pressure, mean arterial pressure, and heart rate in ovariectomized rats in the estrogen-receiving and the control groups.

Variable	Control mean ± SEM	Estrogen mean ± SEM	P-value
Systole	107±7.1	103±8.2	0.0759
Diastole	76±5.6	74±5.01	0.0657
Mean arterial pressure	83.6±6.9	83.6±6.9	0.0839
Heart rate	285±14	274±13	0.1596

The above factors were compared between the estrogen and the control groups using paired t-test

both groups before infusion of PE; namely, just prior to the condition of acute hypertension, both groups of rats were hemodynamically normal and identical in terms of HR.

As may be seen on **Figure 2**, the baroreflex sensitivity of ovariectomized rats enhanced from 0.51 ± 0.06 to 1.52 ± 0.26 in the estrogen-receiving group, indicating an increased sensitivity of baroreflex sensitivity in the estrogen-receiving group compared with the control group ($p=0.009$). **Figure 3** depicts that the BRS of the ovariectomized rats in the control group (receiving sesame oil) decreased from 0.52 ± 0.07 in the saline receiving group and 0.63 ± 0.08 in the propranolol

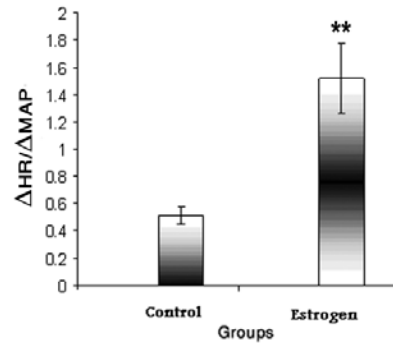


Figure 2 - Comparison of the baroreflex sensitivity ($\Delta HR/\Delta MAP$) between the control group and the case group (receiving estrogen) in ovariectomized rats. $**p=0.009$. HR - heart rate, MAP - mean arterial pressure

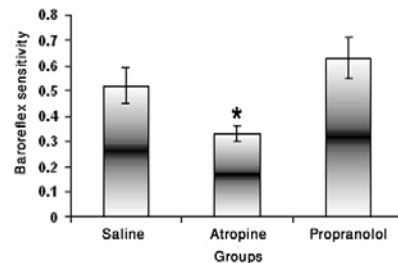


Figure 3 - Comparison of the baroreflex sensitivity in the control group (saline microinjection) versus the propranolol and atropine groups in the nucleus tractus solitarii of ovariectomized rats. $*p=0.0457$

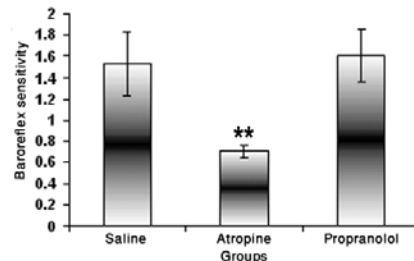


Figure 4 - Comparison of the baroreflex sensitivity in the estrogen-receiving group (saline microinjection) versus the propranolol and atropine groups in the nucleus tractus solitarii of ovariectomized rats. $*p=0.0084$

receiving group to 0.33 ± 0.03 in the atropine-receiving group, indicating that in the ovariectomized rats of the control group, BRS decreases after infusion of atropine compared to the infusion of propranolol and saline ($p=0.0457$). **Figure 4** indicates that BRS in the estrogen-receiving ovariectomized rats decreases from 1.61 ± 0.2 in the saline receiving group and 1.65 in the propranolol-receiving group to 0.8 ± 0.09 in the atropine-receiving group. Thus, the BRS of the estrogen-receiving

ovariectomized rats decreases after infusion of atropine compared with the infusion of saline and propranolol ($p=0.0084$).

Discussion. Our study is one step towards resolving cardiovascular problems through sex hormones. In this study, we indicated systolic and diastolic blood pressure, as well as the MAP and HR to be similar in ovariectomized rats prior to PE injection in both groups receiving estrogen or sesame oil; in other words, before the conditions of acute hypertension, all rats in the estrogen or sesame oil receiving groups were hemodynamically normal and similar in terms of MAP and HR. Following the injection of PE (16 $\mu\text{g}/\text{kg}$), the change in HR decreased in the estrogen-receiving groups compared with the control group.

In the estrogen-receiving group, infusion of PE with the same dose resulted in severe decrease of heart rate; in other words, estrogen intensifies the bradycardia reflex caused by PE in rats. The change in HR was different between the estrogen group and the control group. Following the infusion of PE and establishment of acute hypertension, the MAP increased in the control group. In the estrogen-receiving group, the same scenario caused a decrease in MAP and the change in MAP was decreased in the estrogen group compared with the control group. Therefore, estrogen suppresses dramatic changes of MAP under acute conditions and prevents its abrupt rise; a finding that is in line with that of Saleh and Connell¹⁵ who injected estrogen into the central nucleus amygdala to increase the baroreceptor reflex. According to previous studies, estrogen enhances the BRS considerably,¹⁶ and induces resistance against autonomic disorders.³⁵

In the present study, the BRS is considerably higher in the estrogen-receiving ovariectomized rats compared with the group receiving sesame oil. We compared the BRS in each of the control and estrogen-receiving groups under 3 conditions of atropine, propranolol, and saline microinjection into the NTS to discover that BRS in the estrogen group is considerably higher compared with the group receiving sesame oil. Baroreflex sensitivity has been widely accepted as a tool for clinically diagnosing people at risk of cardiovascular diseases. It has been demonstrated clinically that women with cardiovascular diseases have considerably lower serum levels of estrogen compared with healthy women of similar ages.³⁶ Even in various stages of the menstrual cycle and pregnancy, the autonomic tone and the reflex controlling the autonomic function depend on the plasma levels of estrogen.³⁷ In a study by Goldman et al¹⁸ a single dose

of estrogen caused significant changes in BRS. In other studies, supplementary estrogen reduced blood pressure by decreasing the activity of the renal nerve, dilating the vessels, and reducing stress-induced hypertension.³⁸ Following acute estrogen induction, BRS decreases as well as the sympathetic tone.³⁷

Estrogen injection into other cerebral nuclei, such as the PBN, induces changes in autonomic and cardiovascular parameters. It particularly reduces MAP, HR and the sympathetic tone while increasing the parasympathetic tone. Microinjection of 17- β estradiol into the PBN suppresses the membrane stimulation and modifies the transmission of visceral signals to the ventrobasal nucleus of thalamus, inducing its autonomic effects.^{17,39} We observed the effect of estrogen intensifying the bradycardia reflex caused by PE. We also noticed that estrogen enhances the BRS through the autonomic system in the NTS, particularly the parasympathetic system.

One advantage of the present study is the subcutaneously implanted capsule of estrogen, which releases estrogen to the bloodstream with a constant dose, acting like a natural gland. Previous studies, however, used subcutaneous or intra-peritoneal injection of estrogen, which results in reduction of the hormone concentration over time without maintaining a constant level in bloodstream. The findings of the present study indicate that the NTS affects the BRS through the autonomic system. It may also be concluded that estrogen enhances the bradycardia reflex and inhibits the rise in MAP "under conditions of acute hypertension" as well as the increased BRS in the estrogen group compared with the control group.

Our findings also indicate that the effects of NTS on BRS are primarily exerted through the parasympathetic system, since atropine reduced BRS via the muscarinic receptors and inhibition of the parasympathetic system. Atropine microinjection in the NTS reduced the BRS, whereas propranolol or saline microinjection had no effect on BRS. Therefore, NTS does not affect the BRS through the sympathetic system and the role of β -adrenergic receptors is minimal in this region, while the presence and role of muscarinic receptors are greater in the NTS.

The findings of this study are consistent with those of our previous study on male rats, indicating similar mechanisms in both genders of the animal, thus highlighting the values of hormone therapy.³⁴

In conclusion, it is probable that estrogen affects the NTS via the parasympathetic system under conditions of acute hypertension, leading to increased BRS.

These findings may indicate the advantage of estrogen therapy/hormone therapy. Therefore, it is essential to conduct further studies on the autonomic system in other cerebral nuclei.

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