Effects of Estrogen on Blood Pressure and Heart Rate Responses to L-NAME Treatment in Ovariectomized Rats

L-NAME Uygulanmış Overektomize Sıçanlarda Östrojenin, Kan Basıncı ve Kalp Hızı Yanıtları Üzerine Etkisi

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Abstract
Purpose: This study was to evaluate whether the effect of estradiol on the blood pressure and heart rate responses to acute inhibition of nitric oxide synthase in female Wistar rats.
Material and Methods: Rats were divided into five groups consisting of seven rats each; 1. Sham-operated age-control (Sham); 2. Ovariectomized (O); 3. Ovariectomized plus estradiol (OE); 4. Ovariectomized plus L-nitro-arginine methyl ester, an inhibitor of nitric oxide synthase (OL-NAME);5. Ovariectomized plus estradiol and nitric oxide synthase (OEL-NAME).
Results: Mean, systolic and diastolic arterial blood pressures in sham, ovariectomized and ovariectomized plus estradiol were at close levels to each other. Mean, systolic and diastolic arterial blood pressures increased significantly in the ovariectomized plus estradiol and L-NAME tretment group relative to ovariectomized plus L-NAME treatment group.
Conclusion: This study has showed that estradiol does not play a significant role in modulating the blood pressure of female rats

Key words: **Blood pressure; Estradiol; Heart rate; L-NAME; Nitric oxide; Ovariectomy.**

Özet
Amaç: Bu çalışmada nitrik oksit inhibisyonu yapılmış dişi Wistar sıçanlarda östrodiolün kan basıncı ve kalp hızına etkisinin olup olmadığının araştırılması amaçlanmıştır.
Gereç ve Yöntem: Sıçanlar her grubta yedi hayvan olacak şekilde, aynı yaş grubu kontrol (Sham), ovarektomize (O) , ovarektomize ve östradiol verilen (OE), ovarektomize ve nitrik oksit sentaz enzim inhibitörü L-nitro-arjinin metil ester verilen (O L-NAME), ovarektomize, östradiol ve L-nitro-arjinin metil ester verilen grup olmak üzere (OE L-NAME) beş gruba ayrılmıştır.
Bulgular: Sistolik, diyastolik ve ortalama arteryal kan basınçları sham, sadece ovarektomize ve ovarektomize edilip östradiol verilen gruplarda birbirine yakın değerlerde bulunmuştur. Ortalama, sistolik ve diyastolik arteryal basınçlar ovarektomize edilip östradiol ve L-NAME verilen grupta, ovarektomize ve L-NAME verilen grup değerlerinden istatistiksel anlamda yüksek bulunmuştur.
Sonuç: Dişi sıçanlarda yapılan bu çalışmada östrojenin kan basıncı üzerine önemli bir etkisinin olmadığı belirlenmiştir.

Anahtar kelimeler: **Estradiol; Kan basıncı; Kalp hızı; L-NAME; Nitrik oksit; Ovarektomi.**

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Introduction

Cardiovascular protective effects of estrogen could come partly from the lipid lowering effect of estrogen (1) but this does not seem to explain all the potential cardiovascular benefit (2). Endothelium dysfunction is considered a starting point for atherosclerosis, and thus the endothelium is potentially a major site for protective actions of estrogens (3). Estrogens restore normal endothelial function in humans and animals with atherosclerosis (4). The mechanisms whereby estrogen exerts its effects on the endothelium are not completely understood. Several studies have suggested that a modulation in endothelial nitric oxide synthase (eNOS) expression and in NO generation may be responsible for the beneficial effects of estrogen on endothelial function (5). However, it is currently speculated that it is essential hypertension, the endothelial dysfunction is evoked not by a decrease in NO generation, but by a decrease bioavailability of NO (6).

Both oxygen and nitrogen derisatives are involved in the regulation of blood pressure and heart rate (7,8). Several studies have shown that 17β -estradiol reduces lipid peroxidation in plasma and platelet membranes from postmenopausal women (9,10). These results suggest that impairment of endothelium-dependent vasodilation in absence of estrogen could be at least partly related to the production of oxygen-derived free radicals, which inactivate NO (11). Acute or chronic administration of NOS inhibitors has been shown to cause blood pressure elevation and changes in heart rate in normal rats (12,13). However the alterations in the cardiovascular system associated with acut NO-deficient hypertension and changes in blood pressure, heart rate in the ovariectomized and estrogen replacement rats are not completely understood. This study examined the effect of ovariectomy on blood pressure and heart rate, and the potential role of estrogen on these parameters under the condition of NOS inhibition.

Meterial and methods

This study was performed on total 42 female Wistar rats (12 week old and weighing 190-to-240 g). They were housed with free access to food and tap water in a room with controlled temperature (24 ^{o}C) and lighting. The animals were randomly divided into five groups of seven rats each: Sham-operated age-control (Sham), ovariectomized (O), ovariectomized plus estradiol (OE), ovariectomized plus L-nitro-arginine methyl ester, an inhibitor of NO synthase treatment (OL-NAME) and ovariecthomized plus estradiol and L-NAME (OEL-NAME) groups. The rats were anesthetized with ketamine (1.2mg/kg) and a midline abdominal incision was made. Then, the ovaries were removed in the rats except Sham operated group. The animals were given kloramfenicol on the day of surgery. Twenty three days after surgery, two groups of ovariectomized (OE and OEL-NAME) received by intramuscular injection 17β-estradiol benzoat (20μg/day) suspended in corn oil as a vehicle once a day for 21 days. The rats in the Sham group received only corn oil. L- NAME was administrated by intraperitoneal injection during the last three days of the experiment (40 mg/kg) in the OL-NAME and OEL-NAME groups.

All rats were anesthetized with a mixture (0.4 ml/kg) of Fentanyl (50 g/ml, 5ml) and Midazolam (5 mg/ml, 2ml) on day 23. Catheters (PE-50) were inserted into femoral arter for the measurement of mean arterial blood pressure (MABP), systolic arterial blood pressure (SBP) and diastolic (DBP) arterial blood pressure. All catheters were filled with heparinized normal saline. The body temperature was maintained at 37C. Two hours after the anesthesia, MABP, SBP and DBP were recorded with a pressure transducer (Nihon Kohden Model-TP-101T) connected to a polygraphic system (Nihon kohden model, RM 6000). The heart rate was counted from the upstroke of the arterial pulse pressure. All experimental procedures were approved by Ethics Comittee of Erciyes University.

The blood samples were allowed to clot for 30 min and this was followed by centrifugation at 3000 rpm for 5 min to seperate the serum samples which were stored at -20 ^{o}C. Serum concentrations of estradiol were measured using ^{125}I-labeled radioimmuneassay kit (Diagnostic Products, Texas, USA). The intraassay and interassay coefficients of variation are 8.9 and 7.3% respectively, and the limit of detection of the assay is from 5.4 to 374 pg/ml. All samples, including the standart curve, were run in duplicate and the average is reported. Serum concentrations of total cholestrol (T chol), high-density lipoprotein (HDL), and triglyceride (TG) were measured by using an automated analytical system (Konelab 60i, Intelligent Diagnostic Systems, Espoo, Finland). All statistical analyses were performed by using Sigma Stad TM 10.0 (SPSS Inc., Chicago, IL, USA). The normality of distribution was checked. The data were normally disturbed, statistical evaluation was performed by using ANOVA test (Kruskal-Wallis). Statistical significances were accepted only when $p<0.05$.

Results

Animals'weights, estradiol and lipid levels. Sham-operated and O animals gained weight (1.47 % and 7.46% respectively) during the 3- week treatment period. However, the estrogen replacement group did not. The concentration of serum estradiol significantly decreased in ovariectomized group relative to Sham operated group. Estrogen replacement therapy increased the estradiol level in OE group relative to the O group but this increased level was higher than physiological levels (O-Sham; $p<0.04$, O-OE; $p<0.001$, Sham-OE; $p<0.001$, Table 1). Serum total cholestrol levels in OE were higher than those of Sham-operated control ($p<0.001$). HDL in O and OE was also higher than those of Sham-operated control ($p<0.001$). Trigliceride in the O group was higher than those of other two groups but they were not statistically significant.

Baseline Cardiyovascular Variables. Diastolic and mean arterial blood pressures in Sham, O, OE were at close levels to each other but systolic blood pressure in OE was higher than others (Table II, $p<0.02$). MABP; SBP and DBP increased significantly in the ovariectomized plus estradiol and L-NAME tretment group (OEL-NAME) relative to ovariectomized plus L-NAME treatment group (OL-NAME) ($p<0.01$, $p<0.002$, Table III).

The OE group had a lower HR than the other groups' (Sham-OE,$p<0.05$, Table II). When the rats in the ovariectomized and ovariectomized plus estradiol replacement groups were injected with the L- NAME, the HR increased in the L-NAME relative to the ovariectomized plus estradiol and L-NAME tretment group but it was not statistically signeficant(Table III).

Table I . Weight, plasma estrogen status and lipid levels in each group.

Group	Weight (gr)		Estrogen	Total chollesterol	HDL	TG
	Initial	After 24 d	(pg/ml)	(mg/dl)	(mg/dl)	(mg/dl)
C	204 ±13.6	207± 9.7	11.01 ± 1.9	58.1 ± 7.1	37.71 ± 4.6	43.42 ± 11.9
O	201 ± 8.1	216 ± 10.9 [a]	5.46 ± 0.9[a]	66.6 ± 20.8	58.60 ± 11.6[a]	57.00 ± 11.6
OE	190 ± 15.1	191 ±17.1[b,c]	19.35 ± 4.8[b,c]	85.3 ± 8.6[c]	59.23 ± 11.8[c]	54.00 ±16.7

Values are means ± SD, n:7 for each group. O: ovariectomy, E: estrogen, HDL: high-density lipoprotein, TG: triglyceride. C: Sham-operated age-control, O: Ovariectomized, OE: Ovariectomized plus estradiol
a: Significantly different from the C, ($p<0.04$),
b: Significantly different from the O, ($p<0.001$),
c: Significantly different from the C, ($p<0.002$).

Table II . Effects of 17β-estradiol on Heart rate, Systolic, Diastolic and Mean arterial Blood Pressures.

Groups	Heart rate (beats/min)	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Mean arterial Pressure (mmHg)
C	349 ± 44	120 ± 12	77 ± 12	100 ± 16
O	353 ± 28	120 ± 21	98 ± 22	110 ± 18
OE	258 ± 69[a]	118 ± 11[b]	9 2± 06	104 ± 08

Values are means ±SD, n:7 for each group.
a: Significantly different from the C, ($p<0.05$),
b: Significantly different from the C, ($p<0.02$),

Table III . Effects of L-NAME on Heart rate, Systolic, Diastolic and Mean arterial Blood Pressures.

Groups	Heart rate (beats/min)	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Mean arterial Pressure (mmHg)
OL	419± 40	139±15 [a]	97 ±11[b]	114± 09[b]
OEL-NAME	370 ± 26	172±13	137± 05	150± 10

Values are means SD, n:7 for each group.
Ovariectomized plus L-nitro- arginine methyl ester, a blocker of nitric oxide synthase (OL-NAME), Ovariectomized plus estradiol and L-NAME (OEL-NAME).
a: Significantly different from the OEL-NAME, ($p<0.01$),
b: Significantly different from the OEL-NAME, ($p<0.002$).

Discussion

Recent trials of the use of hormone replacement therapy for primary (14,15) and secondary prevention (16,17) of cardiovascular disease have failed to find a benefit, yet in many observational studies from multiple countries, estrogen use with or without progesterone is associated with a decrease in the incidence of cardiovascular disease (CVD) by as much as 50% (4,18). This study has demonstrated that estrogen doesn't play a significant role in modulating the blood pressure of female rats. Our results have showed that there have been no differences in the SBP, DBP, and MABP among the Sham-operated control, ovariectomized and ovariectomized plus estradiol replacement groups of rats. Furthermore, Nickening et al reported no significant differences in blood pressure between ovariectomized or Sham-operated Wistar-Kyoto rats (19). Fadel PJ et al reported that in blood pressure in overiectomized rats has not elevated compared with control. Acute infusion of 17β -estradiol (60-day timed-release pellets containing 1.5 mg/pellet) had no significant effects on arterial pressure in overiectomized rats (20). It was reported that hormone replacement therapy (HRT) did not confer vasculoprotection event early after the start of HRT (21). One possible explanation is that the onset of endothelial dysfunction is too late for disease reversal by estrogen administration. Once vascular lesion occurs, estrogen administration would not be able to reverse to disease process becasuse of the loss of interaction with its receptors and subsequently diminish bioavailable NO (22).

Zicha J et al showed that chronic (40 mg/Kg of body mass per day, administered in the drink fluit for 4 weeks) administration of L-NAME caused similar blood pressure elevation in young and adult rats (18% in young and 20% in adult) when compared to controls (23). We speculated that administration of L-NAME to rats decreased NO synthesis therfore the decrease of NO has been shown to cause vasoconstriction and decrease in blood flow leading to hypoxia/ ischemia by inhibiting tissue respiration enhanced inflammatory reactions and lipid peroxidation in tissues (24- 27). In our study, the interaction of estradiol replacement and L-NAME administration to ovariectomyzed rats caused the additional elevation in blood pressure compared with L-NAME administrated alone in O rats. A decrease in NO concentration by L-NAME may be responsible for the an increase in superoxide concentration. We theorized that in vivo lipid peroxidation might be increased when circulating estradiol concentrations above the physiological limits in L-NAME hypertensive rats. Interestingly, it has been demonstrated in healthy human female subjects that in vivo lipid peroxidation might be increased when circulating estradiol concentrations are below (50pg/ml) or above (300 pg/ml) the physiological limits (28). Studies of Eybl V et al investigated the effect of estradiol on the oxidative damage determined in the liver of rats treated with dimethylarsinic acid that is used as a herbicid, and induces of oxidative damage in various tissues. They demonstrated that arsenic enhanced lipid peroxidation in the liver and estradiol potentiated this effect of arsenic (29). In our study, estradiol replacement may be aggravated the reactive oxygen species after NO withdrawl. The increased oxidative stress in these animals provides a potential explanation for the exacerbation of endothelial dysfunction.

On the other hand, Hernandez and coworker have suggested that a role for angiotensin II has in hemodynamic responses to L-NAME, because L-NAME increased vascular resistance by 186%, whereas in rats pretreated

with losartan, NOS blockade increased total peripheral resistance (TPR) by only 90% (30). As it was seen that one of the key functions of NO is to buffer the action of vasoconstrictors and preserve the tissular perfusion. Recently, Harvey and coworker have reported that in normotensive postmenopausal women, estradiol increases angiotensin II, but not aldosterone at rest (31). This may contribute to higher cardiovascular event rates reported in recent ERT trials.

Low cardiac NO levels have been shown to induce positive contractile response of the heart leading to increase in HR (25,32,33). NO can regulate both adenylyl cyclase and guanylyl cyclase in the heart (34). Nevertheless L-NAME administration has also been shown to decrease heart rate or no significant change (33,35). The discrepancies between the results of studies that explored the effect of L-NAME may be due to dose, duration, route of administration, metabolism of the drug and animal species used. The heart rate in normal rats is very high when compared to other animal species. L-NAME is more active when given orally to rats. Interestingly, in this study, HR increased with L-NAME administrated ovarectomized rats, estradiol replacement decreased HR but not significant. Contrary, blood pressure increased in ovarectomized plus estradiol and L-NAME treatment group. It is difficult to explain this contradictory between blood pressure and HR responses in estradiol treatment and NO inhibition.

In summary, our study has showed that estradiol replacement and acute L-NAME administration to rats caused the significant elevation in blood pressure but the do not change the heart rate. Estrogen does not provide considerable protection against oxidative injury under NOS inhibition.

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