

Original article

THE ACUTE RELAXANT EFFECTS OF ESTROGEN RECEPTOR AGONISTS IN DIABETIC–OVARIECTOMIZED RAT AORTA

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**Abstract**

Estrogen has protective effects against cardiovascular disease in pre-menopausal women which are decreased in diabetes and menopause. ER- $\alpha$  and ER- $\beta$  mediate estrogen effects, but acute effects on vasculature are not completely understood. In this study, we investigated the contribution of estrogen receptors (ERs)  $\alpha$  and  $\beta$  to vasorelaxation in thoracic aorta rings from control (C), diabetic (D), ovariectomized (O) and ovariectomized-diabetic (OD) rats. Diabetes was induced with streptozotocin (45mg/kg,iv.) and ovariectomy was performed with bilaterally operation. The experimental duration was 8 weeks. Direct relaxant effects of the selective ER agonists 4,4'-(4-propyl-[<sup>3</sup>H]pyrazole-1,3,5-triyl)tris-phenol (PPT), 2,3-bis(4-hydroxyphenyl)-propionitril (DPN) and the nonselective ER agonist 17 $\beta$ -estradiol (E2) were determined after precontracted with phenylephrine. E2 and PPT produced dose-dependent relaxation ( $10^{-13}$ M- $10^{-7}$ M) with a similar profile. However, the maximal relaxation ( $E_{max}$ ) to PPT was higher than that of E2 and was diminished in aorta from O and D groups, furthermore was abolished in aorta from OD group.  $E_{max}$  to DPN was weakened in all aortic groups in comparison with C group. These findings suggest that there is a predominant role of ER- $\alpha$  on estrogen-induced vasorelaxation which is altered along with hormonal status. Hence, the ER- $\alpha$  agonists may offer a new treatment option for the protection of cardiovascular disease.

**Key words:** Estrogen receptor  $\alpha$ , Estrogen receptor  $\beta$ , Ovariectomy, Diabetes, Rat, Aorta.

**Estrojen Reseptör Agonistlerinin Ovariectomize-diyabetik Sıçan Aortasındaki Akut Gevşetici Etkileri**

Estrojen premenapozal kadınlarda kardiyovasküler hastalıkların gelişimine karşı koruyucu etki oluştursa da bu etki diyabet ve menapoz ile azalmaktadır. Estrojen damar üzerindeki etkilerini ER- $\alpha$  ve ER- $\beta$  olmak üzere iki farklı reseptör aracılığı ile yapmaktadır, ancak bu reseptörlerin aracılık ettiği akut etkiler çok iyi bilinmemektedir. Bu çalışmada, kontrol (C), diyabetik (D), ovariectomize (O) ve ovariectomize-diyabetik (OD) sıçanların torasik aortalarında estrojen reseptörlerinin gevşeme yanıtlarındaki rolü araştırılmıştır. Diyabet streptozotocin ile (45mg/kg, i.v.), ovariectomi ise bilateral operasyon ile yapılmıştır. Deney süresi 8 haftadır. Endoteli intakt aorta halkaları organ banyosuna yerleştirilerek fenilefrin ile önkastrıldıktan sonra, selektif ER $\alpha$  agonist 4,4'-(4-propyl-[<sup>3</sup>H]pyrazole-1,3,5-triyl)tris-phenol (PPT), ER- $\beta$  agonist 2,3-bis(4-hydroxyphenyl)-propionitril (DPN) ve selektif olmayan ER agonist 17 $\beta$  estradiolün (E2) gevşeme yanıtları incelenmiştir. Tüm agonistlerin doz-bağımlı ( $10^{-13}$ M- $10^{-7}$ M) gevşeme oluşturdıkları saptanmıştır. PPT ve E2 benzer gevşeme profili göstermiştir. PPT ile oluşan maksimum gevşeme yanıtlarının ( $E_{maks}$ ), E2 yanıtlarından daha fazla olduğu bulunmuştur. Bu yanıtların D ve O gruplarındaki aortalarda azaldığı ve OD grubunda ise tümüyle ortadan kalktığı görülmüştür. Kontrol grubu ile karşılaştırıldığında, DPN ile elde edilen  $E_{maks}$  yanıtlarının daha zayıf olduğu dikkat çekmiştir. Bu çalışmanın sonuçları, ER- $\alpha$ 'nın estrojenle oluşturulan gevşeme yanıtlarında baskın bir rolü olduğunu ve bu etkinin hormonal duruma göre değiştiğini göstermektedir. Böylece, ER $\alpha$  agonistleri kardiyovasküler hastalıklardan korunmada yeni bir tedavi yaklaşımı olarak değerlendirilebilir.

**Anahtar kelimeler:** Estrojen reseptör  $\alpha$ , Estrojen reseptör  $\beta$ , Ovariectomi, Diyabet, Sıçan, Aort.

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## INTRODUCTION

Epidemiological studies suggest that the incidence of cardiovascular disease is lower among premenopausal women aged 35-45 years old compared with men of similar age and post menopausal women (1,2). With increasing lifetime around the globe, the menopause may be considered to be a mid-life event (2). Diabetes mellitus is also the most common chronic disease, which is leading cause of death just over half of whom are women (4). Both diabetes and estrogen deficiency are major constituents of the rise in cardiovascular diseases associated with age, while women in the premenopausal period tend to have much lower rates of the cardiovascular disease compared to the men (5,6). Furthermore these beneficial effects are not evident in women with diabetes (7,8).

The cardiovascular protective effect of estrogen has been shown in several experimental and observative studies (9,10,11). Estrogen reduced cardiovascular disease through multiple mechanisms including favorable effects on plasma lipoproteins, antioxidant effects, preservations of vascular endothelium function, upregulation of nitric oxide (NO) synthase, enhances NO production, inhibits platelet aggregation, increases prostacyclin production, decreases cell adhesion molecules and inflammatory factors (8,12). Although several randomized clinical studies and meta- analyses have indicated that estrogen in post-menopausal women does not affect the incidence of cardiovascular diseases. Moreover it increases triglyceride levels and the risk of stroke (8). The mechanisms of the existence of conflicting data about estrogen remain unclear.

The cellular responses to estrogens are mediated by interactions with either nuclear –or membrane located estrogen receptor (ER- $\alpha$  or ER- $\beta$ ) which the expression in the vasculature is highly regulated both by endocrine status and pathological conditions (6, 13,14,15). Circulating hormones not only differentially modulate the ER isoforms but also mediate distinct actions in vasculature (9,16,17). Recently, Bolego et al. demonstrated that ER- $\beta$  selective agonist induces acute vascular relaxation in aorta from intact rats (10). This effect involves in the increase in NO production (17). Same investigators also observed that the acute vasodilatation to both nonselective ER and ER- $\alpha$  selective agonists in aortic rings was abolished from ovariectomized rats. But it is not known whether the contribution of the estrogen receptors in the vascular system are altered in diabetes and diabetic-ovariectomized rats. Therefore the aim of the present study was to examine the acute relaxant effects of estrogen receptor agonists on thoracic aorta from diabetic-ovariectomized rats.

## EXPERIMENTAL

### *Materials and methods*

Female Wistar rats weighing 200-220 g were housed in Experimental Animal Unit of Faculty of Pharmacy, Ankara University. The animals were kept in temperature controlled facilities on 12-h light/dark cycle and freely accessed food and water. The rats were separated into 4 groups as control (C), diabetic (D), ovariectomized (O) and ovariectomized-diabetic (OD) groups (Ethic committee decision of Medical faculty of Ankara University N 1052745, 8.01.2007). During 8 weeks, body weight and blood glucose level of all groups were measured once in a two-week. After 8 weeks, rats were sacrificed with ketamine (40 mg/kg).

### *Ovariectomy and diabetes induction*

Bilateral ovariectomy was performed under ketamine+xylazine (40/20  $\mu$ g/kg, i.p.) anesthesia (17). Operation site of the rats was cleaned from hair and applied antiseptic solution

before operation. 1 week later ovariectomy, streptozotocin (STZ) which in citrate tampon (pH 4.5) was applied into the tail vein of D and OD groups doses at 45 mg/kg. After 3 days of STZ injection, blood glucose levels was determined by glucometer (Accu check -Go, Bayer) and rats with blood glucose > 250 mg/dl were accepted as diabetic.

Blood samples were collected by cardiac puncture under ketamine anesthesia and centrifuged at 5000 rpm for 5 minutes. Plasma samples was stored in -20 °C until biochemical measurements.

#### *Isolation of the rat thoracic aorta*

After sacrifice of the rat, the thoracic aorta was carefully removed, cleaned of fat and connective tissue, and cut into 3-5 mm rings. The rings were mounted in 10 ml organ chamber which contained standart Krebs' solution and continuously bubbled with 95 %O<sub>2</sub>, %5 CO<sub>2</sub> at 37 °C. The Krebs' solution had the following composition: 118 mM NaCl, 4,7 mM KCl, 1,2mM KH<sub>2</sub>PO<sub>4</sub>, 1.1 mM MgSO<sub>4</sub>, 2.5 mM CaCl<sub>2</sub>, 25 mM NaHCO<sub>3</sub> and 5.5 mM glucose (pH 7.4). The rings were connected to isometric tension transducers (May, Turkey) which coupled with digital data recording system (Biopak, MP30 software; May, Turkey). The rings were equilibrated for 60 min, washed with Krebs' solution every 15 minutes. Experiments were carried out on rings precontracted with phenylephrine (PE) to 60% of maximal contraction (EC<sub>60</sub>: 10 µM). The endothelium was considered functional when relaxation of precontracted rings to 10 µM acetylcholine (Ach) was at least 70% on precontracted rings.

#### *The experimental protocols*

The cumulative concentration-response curves of 17β-estradiol (E<sub>2</sub>; non-selective ligand), PPT (ER-α agonist), and DPN (ER-β agonist) were obtained on PE- precontracted aortic rings. Relaxation responses were expressed as a percentage of residual PE contraction. We also investigated NO-dependent relaxant effects of ER agonists on aortic rings. Thus, aortic preparations were incubated with N (G)-nitro-L- arginine methyl ester (L-NAME) that non-selective NO synthase inhibitors for 20 minutes at 0.1 µM concentration. Cumulative concentration-response curves of ER receptor agonists were obtained before and after L-NAME incubation.

#### *Drugs and chemicals*

PE, Ach chloride, L-NAME and E<sub>2</sub> were purchased from Sigma chemical (USA). PPT and DPN were purchased from Tocris (UK). PE, Ach and L-NAME were dissolved in distilled water, whereas E<sub>2</sub> was freshly dissolved in ethanol to concentration of 10<sup>-3</sup> M. PPT and DPN were also dissolved in ethanol. The final ethanol concentration in organ baths did not exceed 0.01%.

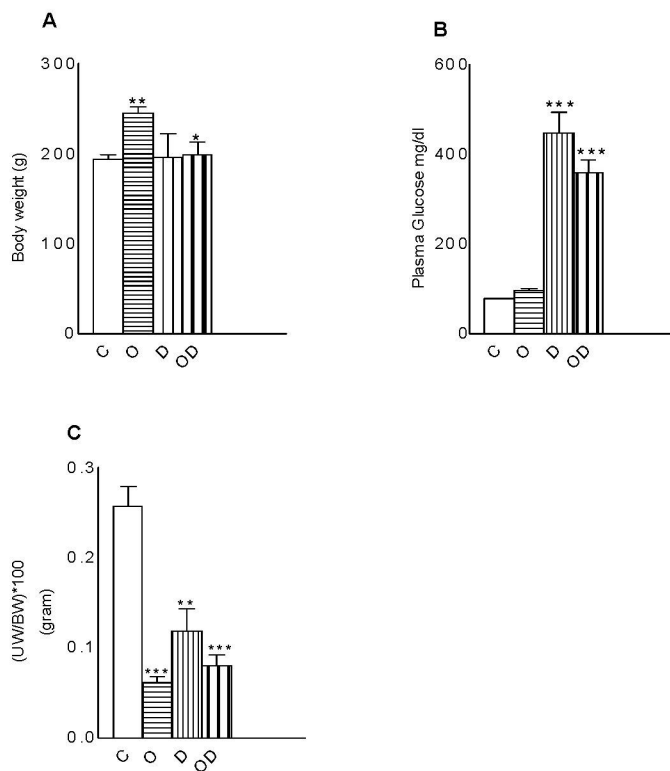
#### *Statistical analysis*

All results of experiments are expressed as meaning ± SEM. Concentration-response curves were obtained with software Prism (Graph Pad Software Inc Sandiego, CA) and compared each concentration-response by means two-way ANOVA followed Bonferroni' post host test. We used one-way ANOVA followed Bonferroni' post host test for all bar graphes performed. Values of p: \*, P<0.05, \*\*, P<0.01 and \*\*\*, P<0.001 were statistically significant compared to C group' #, p<0.5, ##, p<0.01, ###, p< 0.001; compared to PPT responses.

## RESULTS AND DISCUSSION

### *Body weight, uterus weight and blood glucose*

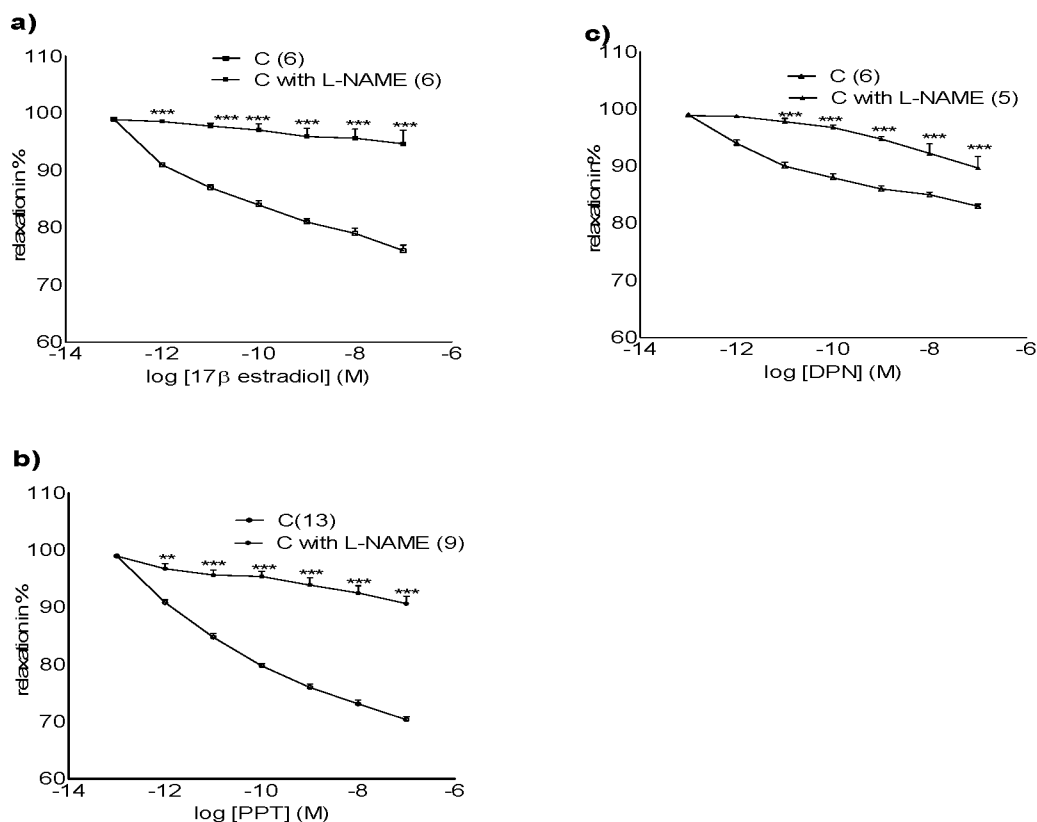
Body weights, uterus weights and blood glucose levels of rats were changed by induction of diabetes and ovariectomy (Figure 1). As expected, blood glucose levels in D and OD groups were increased 3 to 4 fold compared to C group, but was not statistically changed in O group (Figure 1B). Body weight of O group was significantly increased, but body weight of OD group was not changed compared with C group (Figure 1A). A better indicator of persistent estrogen action is related to uterine weight. Thus, we calculated uterus weight to body weight ratio (UA/BA) in all groups at 8 weeks (Figure 1C). UA/BA values of O and OD groups were 3 to 4 fold lower than those of C group. Diabetes induction also decreased uterus weights of D group in a similar fashion.



**Figure 1.** A. Body weight, B. Blood glucose and C. The ratio of uterus weight to body weight (UW / BA) all groups at the end of 8 weeks. C: Control , D: Diabetic, O: Ovariectomized, OD: Ovariectomized-diabetic. Mean  $\pm$  SEM, compared to C group \*  $p < 0.5$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

*Influence of L-NAME incubation on the relaxations induced by 17β-estradiol (E<sub>2</sub>) in aorta from control (C) group.*

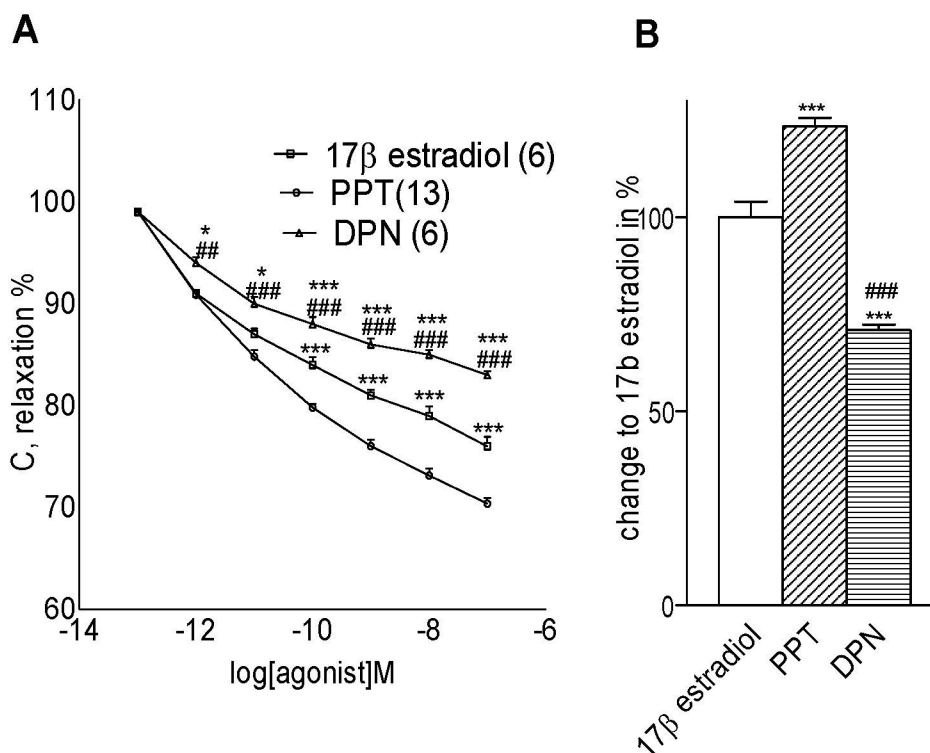
To investigate NO-dependent vasodilatation of E<sub>2</sub>, PPT and DPN in C group, we used non-selective NOS inhibitor L-NAME (0.1 μM). After 20 min incubation period with L-NAME, aorta were precontracted with PE (at concentration produced by 60 % of maximal response) and relaxed by selective and non-selective ER receptor agonists (Figure 2). In the absence of L-NAME, E<sub>max</sub> values of E<sub>2</sub>, PPT and DPN were 24 ± 0.96 %, 30 ± 0.96 %, 17 ± 0.36 %, respectively. After L-NAME incubation, E<sub>max</sub> values were 5.26 ± 2.033 %, 9.33 ± 1.312 %, and 10.32 ± 1.99 %, respectively. Relaxant effects of the agonists were decreased approximately by 80 %. Thus, we found that vasodilator responses induced by all ER agonists were NO- dependent.



**Figure 2.** a) E<sub>2</sub>, b) PPT, c) DPN concentration-response curves with and without L-NAME (0.1 μM) in aorta, precontracted with PE (concentration at 60% of maximal response), from control (C) group. Mean ± SEM, \*\*\*p<0.001.

*Relaxation responses of PPT and DPN in comparison with 17β-estradiol (E<sub>2</sub>) in aorta from control (C) group*

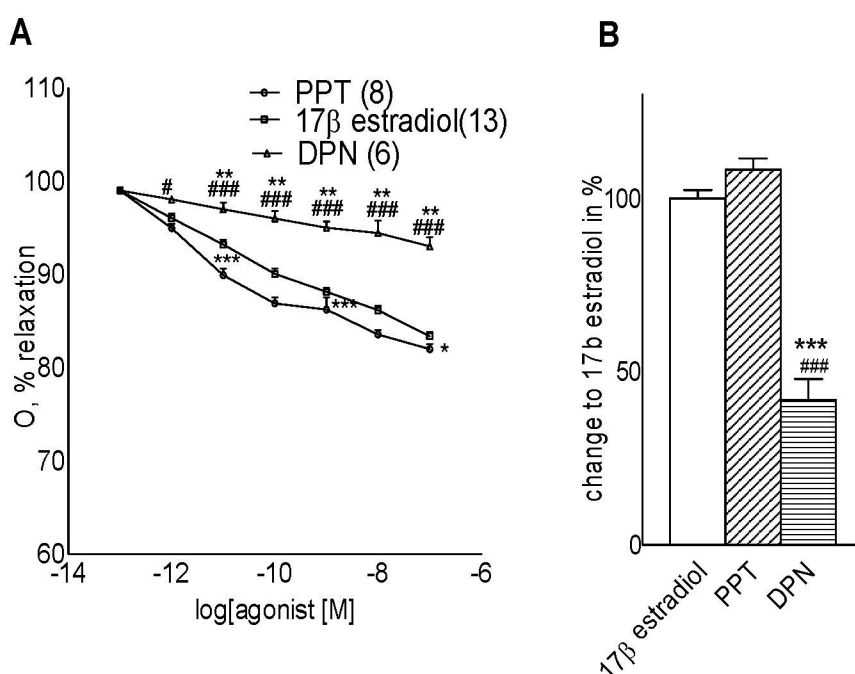
Fig 3A shows concentration-response curves of selective and non-selective agonists in aorta, precontracted with PE (concentration at 60% of maximal response), from C groups. E<sub>max</sub> of ER-α agonist PPT (29,62 ± 0,54%) was higher than that of E<sub>2</sub> (24 ± 0,96%), whereas E<sub>max</sub> of DPN (17 ± 0.36 %) was lower than that of E<sub>2</sub>. % of changes of E<sub>max</sub> values of PPT and DPN compared to E<sub>2</sub> are shown in Figure 3B. Thus; PPT (% of change: +23.4 ± 3) had advantage in terms of the amount of maximal relaxation ratio, but DPN had not (% of change: -30 ± 1.52).



**Figure 3.** A. Concentration-response curves of E<sub>2</sub>, PPT and DPN, in aorta from control group, precontracted with PE. B. Bar graph shows % changes of E<sub>max</sub> values. The parenthesis near the group names are (n) numbers. Mean ± SEM, \*p< 0.5, \*\*\*p<0.001, significant to E<sub>2</sub> response, ## p<0.01, ### p< 0.001; significant to PPT response.

*Relaxation responses of PPT and DPN in comparison with 17 $\beta$ -estradiol (E<sub>2</sub>) in aorta from ovariectomized group*

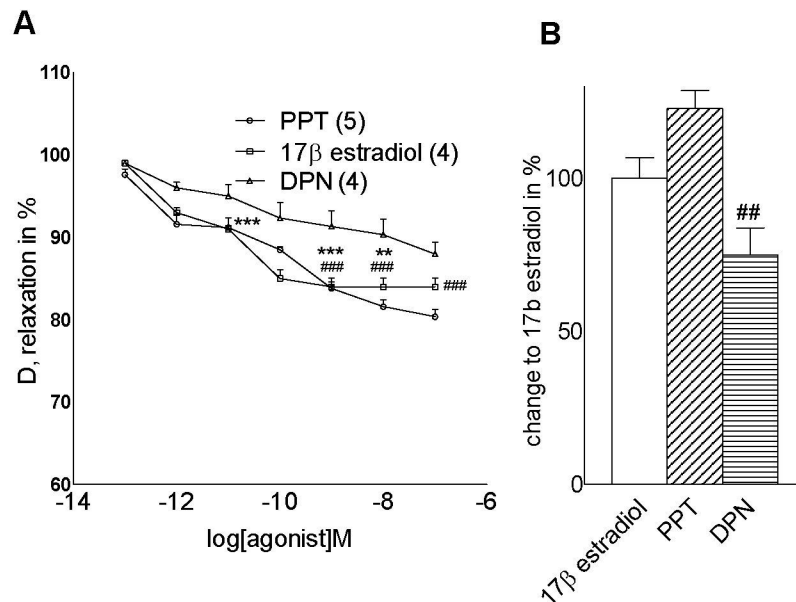
Figure 4A shows cumulative concentration-response curves induced by E<sub>2</sub>, ER $\alpha$ -agonist PPT and ER $\beta$ -agonist DPN in aorta from O group. Emax values of E<sub>2</sub>, PPT and DPN were 16,6  $\pm$  0,42 %, 18  $\pm$  0,53 % 6,7  $\pm$  1.0 %, respectively. On the other hand, Emax value of PPT (108.3  $\pm$  3.23 %) was not changed when compared to E<sub>2</sub>, whereas % change of DPN (- 41.93  $\pm$  6.02%) was significant when compared to E<sub>2</sub>. Thus, we did not find any advantages of relaxant responses induced by PPT and DPN in aorta from O group compared to E<sub>2</sub>. After ovariectomy, ER receptors might be down regulated due to lacking of E<sub>2</sub>. Thus, all relaxant responses in aorta from O group were lower than that of E<sub>2</sub> in aorta from C group. May be normal circulating E<sub>2</sub> levels is required for the normal action of PPT and DPN on vasculature.



**Figure 4.** A. Concentration-response curves of E<sub>2</sub>, PPT and DPN in aorta, precontracted with PE, from ovariectomized (O) group. B. Bar graph shows % change of Emax values. The parenthesis near the group names are shown (n) numbers. Mean  $\pm$  SEM \*p < 0.5, \*\*p < 0.01, \*\*\* p < 0.001, significant to E<sub>2</sub> response. # p < 0.5, ### p < 0.001; significant to PPT response.

*Relaxation responses of PPT and DPN in comparison with 17β-estradiol (E<sub>2</sub>) in aorta from diabetic (D) group*

Figure 5 shows the concentration-response curves induced by E<sub>2</sub>, PPT and DPN in aorta from D group. E<sub>max</sub> value of E<sub>2</sub>, PPT and DPN were 16,0 ± 1.08 %, 19,64 ± 0,94% , 12,0 ± 1.41%, respectively. % relative changes of E<sub>max</sub> for PPT and DPN were +22.7 ± 5.89, and -25 ± 8.84%, respectively, when compared to E<sub>2</sub> response. On the other hand, when compared to E<sub>max</sub> of E<sub>2</sub> in C group, E<sub>max</sub> values of all agonists in aorta from D group were found to be decreased. Thus, ER<sub>α</sub>- agonist PPT had an advantage on vasorelaxation compared to E<sub>2</sub> in diabetic state. However, in diabetes, vasorelaxant effect of selective ER<sub>β</sub>-agonist DPN was lost by half compared with that of E<sub>2</sub>.

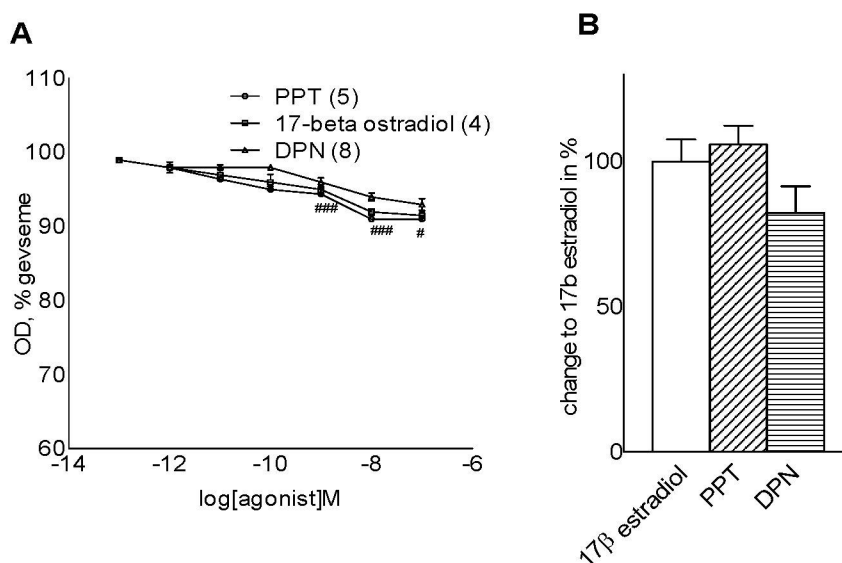


**Figure 5.** A. Concentration-response curves of E<sub>2</sub>, PPT and DPN in PE-precontracted aortic rings from diabetic (D) rats. B. Bar graph compares % changes of E<sub>max</sub> values of PPT and DPN to E<sub>2</sub>. The parenthesis near the group names are (n) numbers. Mean ± SEM, \*\*p<0.01, \*\*\*, p<0.001, significant to E<sub>2</sub> response. ##, p<0.01, ###, p<0.001; significant to PPT response.



*Relaxation responses of PPT and DPN in comparison with E2 in aorta from ovariectomized-diabetic (OD) group*

The concentration-response curves of E2, PPT and DPN in aorta from ovariectomized-diabetic (OD) group are shown in Figure 6A. Emax values of all agonists were decreased approximately by 70% compared to E2 response in C group. Emax values of E2, PPT and DPN were  $8,5 \pm 0,64\%$ ,  $9,0 \pm 0,5\%$ ,  $7,0 \pm 0,78\%$ , respectively. On the other hand, Emax values of three agonist in O and D groups were decreased by 50 % compared to E2 response in C group. Thus, induction of diabetes and ovariectomy could have been synergistic effects on decreasing relaxant responses.



**Figure 6.** A. Concentration-response curves of E2, PPT and DPN in precontracted aorta from ovariectomized-diabetic rats (OD). B. Bar graph compares % change of Emax values of PPT and DPN to E2. The parenthesis near the group names are (n) numbers. Mean  $\pm$  SEM. #,  $p < 0,5$ , ###,  $p < 0,001$ , significant to PPT response

## DISCUSSION

In the present study we have investigated that the acute vasorelaxant effects of specific and nonspecific ER- agonists were decreased in aorta from ovariectomised and diabetic rats. Furthermore, for the first time we are reporting that these responses were completely abolished by combinatory application of diabetes and ovariectomy.

8 week-ovariectomized rats exhibited an increase in body weight compared to C group animals. This can be due to menopausal state (early stage) and declined in ovarian function. The exact mechanism (s) is not completely understood however, it may be related with an altered levels of circulating hormones such as luteinizing hormone and leptin (11,17,19,5). In conversely, diabetic rats exhibited a decrease in body weight compared to C group rats, which may include different mechanisms (8,19). For example, changes in metabolism of fatty acids, proteins and carbohydrates could be important reasons of decreasing in body weight. Thus, ovariectomy and diabetes have controversially effect in terms of body weight.

Attenuation of the uterus weight to the body weight ratio in D group was considered that is not as serious as in that of O group. This finding indicates that the decrease was not actually due to diminished of circulating estradiol levels. As expected, blood glucose levels were increased in D and OD groups. Blood glucose levels in O groups were increased but this did not reach to statistical significance. Similar results were reported in various studies, although some studies suggested that ovariectomized were caused an augmentation in the blood glucose levels (20-22). These discrepancies may depend on the duration of ovariectomization and ageing of experimental animals. We did not found significant alteration on blood glucose levels between C and O rats. May be the duration of estrogen depletion is not enough to raise glucose level in our study.

It is known that estrogen has an important role in the regulation of vascular function. The roles of the two estrogen receptors, ER- $\alpha$  and ER- $\beta$ , have been investigated on certain cell culture studies or in knockout animal models, but few *ex vivo* studies have been performed to explain the roles of vascular estrogen receptors considering on vascular tone modulation (19,21,23). Both ER $\alpha$  and ER $\beta$  subtypes have been demonstrated to mediate the vasodilator effects of estrogens in cardiovascular system. These effects are mediated by genomic as well as nongenomic mechanisms some of which include increases in local NO (24). Nongenomic activation of endothelial NO synthase (NOS) by estrogens via Er- $\alpha$  has been reported in endothelial cells of different vascular bed such as pulmonary artery and bovine aorta (12,16,25). This response is rapid onset, which unlikely mediated via genomic effects and also occurs in low concentrations, hence influences on vascular tone at physiological concentration. Thus, in our study, we used non-selective NOS inhibitor L-NAME to investigate NO-dependent vasodilatation mechanism of E<sub>2</sub>, PPT and DPN in aorta from C group. After incubation of aortic tissues with L-NAME, we determined that the vasorelaxant effects of selective ER receptor agonists and E<sub>2</sub> were lost. Thus, E<sub>2</sub> and selective ER receptor agonists mediated vasodilatations are NO- dependent.

This study has also been shown that PPT, ER- $\alpha$  agonist mediated relaxant effects in precontracted aortic tissue were similar profile with that induced by E<sub>2</sub> in all experimental groups. In the previous studies, it has been indicated that the vasorelaxant effect of non-specific agonist E<sub>2</sub> was changed in healthy female rats at various stages of oestrous cycle, pregnancy and ovariectomy (13,26). The acute effect of PPT on vascular reactivity might be linked to hormonal status as well as circulating E<sub>2</sub> levels. We have observed that the vasorelaxant effect of E<sub>2</sub> was decreased compared with PPT response. This discrepancy may be due to an antagonistic effect of ER- $\beta$  on ER- $\alpha$  as reported in other studies (8,16, 25,27). In the present study we showed that PPT and E<sub>2</sub> partially lost the ability to induce acute vascular relaxation in ovariectomized rats. In agreement with previous studies, E<sub>2</sub> and PPT induced acute relaxation in aorta (17,23). ER- $\beta$  agonist DPN has the lowest effect compared with the other two agonists in our all experimental groups. On the other hand, to determine any advantage of selective ER receptor agonists over E<sub>2</sub>, we estimated percentage of maximal responses (E<sub>max</sub>) of PPT and DPN in O, D and OD groups and compared with that natural agonist E<sub>2</sub> in C groups. E<sub>max</sub> values of three agonists in O and D groups were decreased to 50 % compared with E<sub>2</sub> in C group, whereas E<sub>max</sub> values of all agonists were approximately decreased to 70% in OD group. We can conclude that ER receptors might be down-regulated due to lacking of circulating E<sub>2</sub> levels together with high glucose levels. In some studies, it was found that under physiological glucose levels estrogen increases ER- $\alpha$  levels without significantly affecting ER- $\beta$  levels (21,22). However under high glucose conditions, estrogen decreases ER- $\alpha$  and increases ER- $\beta$  leading to a decrease in the relative ratio of ER- $\alpha$  to ER- $\beta$ . Thus high glucose appears to reverse the estrogen effects on relative expression of its receptors. It has been shown that high glucose reverses the increase in ER- $\alpha$  to ER- $\beta$  level induced by estrogen under physiological conditions. This is a possible mechanism by which the vasculo-protective effects may be abrogated under high glucose conditions (21,27,27). Thus, in our study, diabetes and ovariectomization could

have synergistic effects for decreasing relaxant responsiveness. On the other hand, we found that PPT has approximately 20% advantageous over on natural agonist E2 in C group, which has normal E2 levels. Thus, in vivo conditions, the presence of E2 may be required for normal action of PPT and DPN on vasculature.

On the other hand, the increased oxidative stress plays a key role the pathogenesis of cardiovascular complications such as diabetes and menopause (5,29,29). Furthermore, oxidative stress was shown to up regulate ER- $\beta$  but not ER- $\alpha$ . Previous findings suggested that free radicals particularly altered the vasorelaxation responses of estrogen (5,6,13,29). This could explain why women suffer greater cardiovascular risk in diabetes with menopause.

In conclusion, the main finding of this study is that selective ER- $\alpha$  agonist, unlike selective ER- $\beta$  agonist, induces acute vascular relaxations in both ovariectomized and diabetic state and the relaxant responses are decreased comparing to normal animals. The combination with diabetes and ovariectomy were further decreased the vasorelaxation response to E2 and PPT. This further attenuation may be due to the oxidative stress induced by increased blood glucose and decreased estrogen levels. We can speculate that the subtype-specific estrogen receptor agonists would be useful for a new treatment option for cardiovascular protection in menopause.

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