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17 β -Estradiol Increases Basal but Not Bradykinin-Stimulated Release of Active t-PA in Young Postmenopausal Women

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Abstract

Angiotensin-converting enzyme inhibition potentiates basal and bradykinin-stimulated tissue-type plasminogen activator (t-PA) release to a greater extent in women than in men. This study tested the hypothesis that 17 β -estradiol enhances the effect of angiotensin-converting enzyme inhibition on t-PA release in young postmenopausal women. We conducted a double-blind, prospective, crossover study in 14 young postmenopausal women (mean age 48.2 \pm 2.3 years) who were randomized to receive 17 β -estradiol (1 mg/d) or matching placebo for 4 weeks. At the end of each treatment period, we measured the effect of intraarterial infusion of bradykinin, methacholine, and nitroprusside on forearm blood flow and net t-PA release, before and during intraarterial enalaprilat (0.33 μ g/min/100 mL forearm volume). 17 β -estradiol significantly reduced baseline venous plasminogen activator inhibitor-1 antigen (4.4 \pm 1.4 versus 10.4 \pm 2.5 ng/mL, $P=0.001$) and t-PA antigen (5.5 \pm 0.6 versus 7.5 \pm 1.3 ng/mL, $P=0.022$) compared with placebo. 17 β -estradiol increased basal forearm vascular release of active t-PA compared with placebo (1.2 \pm 0.3 IU/mL/min versus 0.4 \pm 0.1 IU/mL/min respectively, $P=0.032$), without increasing t-PA antigen release ($P=0.761$). Enalaprilat significantly increased basal net t-PA antigen release (from -0.8 ± 1.0 to 3.2 \pm 1.2 ng/min/100 mL, $P=0.012$), but not the release of active t-PA, during either placebo or 17 β -estradiol. Enalaprilat potentiated bradykinin-stimulated vasodilation and t-PA antigen and activity release similarly during placebo and 17 β -estradiol treatment. 17 β -estradiol treatment does not alter the effect of angiotensin-converting enzyme inhibition on basal t-PA antigen or on bradykinin-stimulated t-PA antigen or activity release. 17 β -estradiol increases basal release of active t-PA in young postmenopausal women, consistent with enhanced vascular fibrinolytic function.

Keywords

estradiol; angiotensin-converting enzyme inhibition; postmenopausal women; plasminogen activator; bradykinin

Estrogen deficiency of menopause causes vascular and metabolic changes that increase the risk for cardiovascular disease.^{1,2} In contrast to observational studies that suggest that hormone replacement therapy (HRT) reduces the risk of coronary heart disease (CHD),^{3–5} recent randomized controlled trials indicate that treatment with conjugated estrogen plus progestin increases the risk of pulmonary emboli, CHD, and stroke, whereas treatment with conjugated estrogen alone increases risk of stroke without affecting embolic events or risk of CHD.^{6–8}

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Disclosures
None.

This discrepancy in results has been attributed to the type of HRT used, the timing of intervention of HRT after menopause,^{9,10} or the route of administration.¹¹ For example, the Heart and Estrogen/progestin Replacement Study (HERS)⁸ and the Women's Health Initiative (WHI)^{6,7} study assessed the effect of treatment with conjugated equine estrogens, a mixture of estrogens extracted from horse urine that also contains progestins and androgens, whereas estradiol is the major endogenous estrogen.¹²

Differences in the outcomes of epidemiological studies and clinical trials are best interpreted in the context of an understanding of the biological effects of estrogen. On the one hand, oral HRT increases circulating fibrin split products (FSP), concentrations of inflammatory cytokines, and C-reactive protein.^{13–15} On the other, estradiol exerts many salutary effects on the vasculature, including improving endothelial-dependent vasodilation,¹⁶ decreasing fibrinogen and plasminogen activator inhibitor (PAI)-1 (PAI-1),^{17–19} increasing the secretion of nitric oxide from intact platelets,²⁰ and attenuating platelet-derived growth factor (PDGF) receptor-activated smooth muscle cell migration and proliferation.²¹

HRT decreases circulating t-PA antigen in parallel with PAI-1 antigen, to which it is complexed. Circulating t-PA antigen does not reflect the capacity of the vasculature to release t-PA, however, and the effect of HRT or estrogen on endothelial fibrinolytic function has not been studied extensively. Hoetzer et al¹⁶ observed that acute intraarterial infusion of 17 β -estradiol enhanced bradykinin-stimulated t-PA antigen release. The investigators also reported that bradykinin-stimulated t-PA release was increased in women chronically taking estrogen, but treatment was not randomized.

We have previously demonstrated that angiotensin-converting enzyme (ACE) inhibition increases basal vascular t-PA antigen release in women but not in men.²² In addition, ACE inhibition potentiates bradykinin-stimulated t-PA release to a greater extent in premenopausal women than in postmenopausal women and in either group of women compared with age-matched men,²³ suggesting that estrogen may modulate the effect of ACE inhibition on bradykinin-stimulated t-PA release. This study tested the hypothesis that 17 β -estradiol enhances t-PA release in the presence of ACE inhibition in young postmenopausal women.

Methods

Subjects

Fourteen healthy postmenopausal women participated in the study. Women were defined as postmenopausal if it had been at least 1 year since their last menstruation and if their untreated estradiol concentration was less than 30 pg/mL or their follicle-stimulating hormone (FSH) concentration exceeded 50 mIU/mL. After written informed consent was obtained, all subjects underwent a complete history and physical examination, and an ECG and routine laboratory were obtained. Subjects with renal, pulmonary, endocrine, hematologic, or cardiovascular disease (including hypertension defined as an untreated seated systolic/diastolic blood pressure greater than 140/90) were excluded. All subjects were within 30% of their ideal body weight. Subjects with fasting cholesterol greater than 5.7 mmol/L (220 mg/dL) and smokers were excluded.

Four subjects (or 30% of subjects) were taking HRT, which was washed out for 1 month before the start of the study. Two subjects were taking conjugated estrogens (Premarin), 1 subject was taking transdermal estradiol (Climara), and 1 subject was taking estradiol vaginal tablet (Vagifem). These subjects were taking HRT for relief of postmenopausal symptoms including hot flashes and vaginal dryness.

Experimental Protocol

The study protocol was approved by the Vanderbilt University Institutional Review Board and conducted according to the Declaration of Helsinki. Subjects were randomized to receive either 17β -estradiol (1 mg/d, Watson Pharmaceuticals) or placebo for 4 weeks followed by the first study day (please see Figure S1 at <http://hyper.ahajournals.org>). After the first study day, subjects were crossed-over (placebo or 17β -estradiol) for 4 weeks followed by study day 2. Four subjects completed only 1 study day (2 during 17β -estradiol and 2 during placebo treatment arm). One subject developed a gastrointestinal illness before her second study day. One subject developed bradycardia during arterial line placement, while another developed tachycardia. In the 4th subject we were not able to obtain arterial access on 1 of the study days.

Studies were performed in the morning in a temperature-controlled room. FBF was measured by silastic-in-mercury strain-gauge plethysmography (please see the online data supplement).²⁴ After measurement of baseline forearm blood flow (FBF) and blood sampling, graded doses of sodium nitroprusside (SNP, Gensia Siccior Pharmaceuticals), methacholine (MCH, Pharmaceutical Compounding Center), and bradykinin (Clinalfa AG) were infused in random order. SNP (endothelium-independent control) was infused at 1.6, 3.2, and 6.4 $\mu\text{g}/\text{min}$; MCH (endothelium-dependent, bradykinin-receptor independent control) at 3.2, 6.4, and 12.8 $\mu\text{g}/\text{min}$, and bradykinin at 100, 200, and 400 ng/min. Each dose was infused for 5 minutes and FBF was measured during the last 2 minutes of infusion.

Thirty minutes after administration of these three drugs, baseline measurements were repeated and subjects then received a continuous intraarterial infusion of enalaprilat (Ben Venue Laboratories, Inc) at 0.33 $\mu\text{g}/\text{min}/100$ mL forearm volume. While continuing the infusion of enalaprilat, baseline measurements and infusions of MCH and bradykinin were repeated. Bradykinin doses were reduced to 25, 50, and 100 ng/min during enalaprilat because 2 subjects in a prior study developed transient arm swelling after infusion of higher doses of bradykinin in the presence of enalaprilat.²²

Blood Sampling and Biochemical Assays

After measurement of FBF, simultaneous arterial and venous samples were obtained from the infused arm before and after each dose of MCH and bradykinin. Blood samples were collected on ice and centrifuged immediately, and plasma was stored at -70°C until the time of assay. Please see the data supplement for details regarding analyses.

Statistical Analysis

Data are presented as mean \pm SEM. Because 4 subjects only completed 1 study day we analyzed the data using 2 different methods. First we analyzed paired data measured in the 10 subjects who completed both days. In addition, we analyzed the unpaired data of all 14 subjects who had completed any study day. Because both analyses yielded the same result, we present here the findings of the paired analysis. The effect of agonists on hemodynamic and fibrinolytic variables was determined using a general linear model-repeated measures ANOVA in which the within-subject variables were vasodilator dose and/or drug arm (17β -estradiol or placebo). The effect of enalaprilat on the t-PA response to 100 ng/min bradykinin was determined using a paired *t* test. To determine whether hormonal status (17β -estradiol or placebo) affected sensitivity to exogenous bradykinin, we constructed individual dose response curves in which bradykinin dose was log transformed. The slopes of these individual curves were then compared between treatments. A 2-tailed probability value less than 0.05 was considered statistically significant. Statistical analysis were performed with the statistical package SPSS for Windows (Version 15.0, SPSS) and GraphPad Prism for Windows (Version 4.0, GraphPad).

Results

Effect of 17 β -Estradiol and Enalaprilat on Baseline Characteristics

Subject characteristics at the time of screening are presented in Table S1. There were no significant changes in systolic blood pressure ($P=0.678$), diastolic blood pressure ($P=0.481$), or mean arterial pressure ($P=0.502$) in subjects taking HRT after washout. Also, treatment with 17 β -estradiol in subjects taking HRT before the study had no effect on systolic blood pressure, diastolic blood pressure, or mean arterial pressure. The time between the last normal menstruation and study date was 5.0 ± 1.2 years for women not taking any HRT. During the study, estradiol concentrations were 2-fold higher during estradiol compared with during placebo (53.7 ± 7.5 versus 24.8 ± 2.5 pg/mL, $P=0.017$). Systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate were similar during treatment with placebo and 17 β -estradiol (Table). Baseline FBF and FVR were also similar in the 2 treatment arms. Venous PAI-1 antigen and venous t-PA antigen were significantly decreased during 17 β -estradiol compared with during placebo. However, baseline venous t-PA activity was not significantly different between the 2 treatment arms. Intraarterial administration of enalaprilat did not affect mean arterial pressure, FBF, or FVR during either the 17 β -estradiol or placebo arm (all probability values >0.367).

Effect of 17 β -Estradiol and Enalaprilat on Basal Vascular Net t-PA Release

Basal net t-PA antigen release (Figure 1A) was similar during treatment with 17 β -estradiol or placebo ($P=0.887$). Enalaprilat significantly increased net t-PA antigen release ($P=0.012$ for effect of enalaprilat, $P=0.761$ for placebo versus 17 β -estradiol). Because 17 β -estradiol treatment significantly decreased PAI-1 antigen, we also assessed the effect of 17 β -estradiol and enalaprilat on basal vascular release of active t-PA (Figure 1B). Basal net release of active t-PA was significantly higher during 17 β -estradiol treatment than during placebo treatment (1.2 ± 0.3 IU/mL/min versus 0.4 ± 0.1 IU/mL/min respectively, $P=0.032$). In contrast to the effect of enalaprilat on net t-PA antigen release, enalaprilat did not enhance the net release of active t-PA ($P=0.884$).

Effect of 17 β -Estradiol and Enalaprilat on FBF Response to Exogenous Agonist

Figure 2 illustrates the effect of bradykinin on FBF in the presence and absence of enalaprilat during placebo and 17 β -estradiol treatment. Bradykinin caused a dose-dependant increase in FBF during placebo ($P=0.004$) as well as during 17 β -estradiol ($P=0.002$) treatment, and there was no significant difference in the vasodilator response during the 2 treatment arms ($P=0.155$, Figure 2A). Enalaprilat significantly enhanced bradykinin-stimulated FBF during placebo treatment (from 9.6 ± 2.1 to 19.3 ± 3.0 mL/min/100 mL at 100 ng/min bradykinin, $P<0.001$) as well as during 17 β -estradiol treatment (from 11.2 ± 1.6 to 23.8 ± 4.2 mL/min/100 mL at 100 ng/min bradykinin, $P=0.001$). The magnitude of the effect of enalaprilat was similar during the 2 treatment arms ($P=0.134$, Figure 2B). The slope of the relationship between bradykinin dose and FBF during enalaprilat was not significantly different between placebo and 17 β -estradiol treatment (Figure 2C and 2D, $P=0.274$), indicating that 17 β -estradiol treatment did not enhance the sensitivity of the vasodilator response to exogenous bradykinin.

MCH significantly increased FBF during both placebo ($P=0.005$) and 17 β -estradiol ($P=0.001$) treatment, and there was no significant difference between the 2 treatment arms ($P=0.243$, data not shown). As expected, enalaprilat did not enhance MCH-stimulated FBF during either placebo ($P=0.786$) or 17 β -estradiol ($P=0.118$) treatment. Similarly, SNP (endothelium-independent control) significantly increased FBF during placebo ($P=0.004$) and 17 β -estradiol ($P=0.002$) treatment and the response to SNP was similar during the 2 arms ($P=0.810$, data not shown).

Effect of 17 β -Estradiol and Enalaprilat on t-PA Response to Exogenous Bradykinin

Bradykinin caused a significant dose-dependent increase in t-PA antigen release during both placebo ($P=0.007$) and 17 β -estradiol ($P=0.007$) treatment (please see Figure S2 at <http://hyper.ahajournals.org>). Enalaprilat significantly enhanced bradykinin-stimulated t-PA antigen release during placebo treatment (from 11.9 \pm 4.7 to 217.9 \pm 52.5 ng/min/100 mL at 100 ng/min bradykinin, $P=0.002$) as well as during 17 β -estradiol treatment (from 16.9 \pm 8.1 to 209.0 \pm 59.1 ng/min/100 mL at 100 ng/min bradykinin, $P=0.007$). There was no effect of placebo versus 17 β -estradiol treatment on bradykinin-stimulated t-PA antigen release in the absence ($P=0.929$, Figure S2A) or presence ($P=0.401$, Figure S2 B) of enalaprilat. The slope of the relationship between brady-kinin dose and t-PA antigen release during enalaprilat was not significantly different (Figure S2C and S2D, $P=0.437$) between the 2 treatment arms.

Because 17 β -estradiol treatment decreased PAI-1 antigen, we also assessed the effect of exogenous bradykinin on the release of active t-PA. Enalaprilat significantly enhanced the release of active t-PA during both placebo (from 7.5 \pm 1.9 to 58.3 \pm 14.2 IU/min/100 mL at 100 ng/min bradykinin, $P=0.002$) and 17 β -estradiol treatment (from 6.6 \pm 3.7 to 66.3 \pm 18.0 IU/min/100 mL at 100 ng/min bradykinin, $P=0.007$). As with bradykinin-stimulated t-PA antigen release, there was no effect of 17 β -estradiol treatment on bradykinin-stimulated release of active t-PA in the absence or presence of enalaprilat ($P=0.744$ and $P=0.217$ respectively, Figure 3A and 3B). The slope of the relationship between bradykinin dose and active t-PA release during enalaprilat was not significantly different (Figure 3C and 3D, $P=0.812$) between the 2 treatment arms.

Discussion

This study examined the effect of 17 β -estradiol treatment on vascular fibrinolytic function in the absence and presence of ACE inhibition in young, otherwise healthy postmenopausal women. As previously described,^{17,25} 17 β -estradiol decreased circulating PAI-1 antigen concentrations. ACE inhibition increased basal release of t-PA antigen, but not activity, and enhanced bradykinin-stimulated vasodilation and t-PA antigen and activity release. 17 β -estradiol did not alter these effects of ACE inhibition but enhanced the basal forearm vascular release of active t-PA.

The endogenous fibrinolytic system plays a critical role in maintaining vascular patency. For example, endothelial fibrinolytic capacity, as measured by stimulated t-PA release from the forearm vasculature, predicts the future risk of adverse cardiovascular events in patients with CHD, with individuals with the lowest t-PA release experiencing the highest rate of adverse events.²⁶ In contrast, increased venous t-PA antigen predicts an increased risk of CHD.²⁷ These apparently contradictory findings may be explained by the different physiological determinants of these 2 measures of t-PA. A single measurement of venous t-PA antigen captures both free active t-PA and t-PA bound to PAI-1; because greater than 70% of t-PA circulates bound to PAI-1, increased t-PA antigen usually manifests an increase in PAI-1 rather than an increase in active t-PA.^{28,29} In contrast, measurement of net release of t-PA takes the arteriovenous gradient and blood flow into account and reflects the capacity of the vascular endothelium to release stored t-PA.³⁰ Thus, in the current study 17 β -estradiol decreased venous t-PA antigen in parallel with PAI-1 antigen without affecting peripheral t-PA activity. Moreover, 17 β -estradiol increased basal forearm vascular release of active t-PA, consistent with a beneficial effect on endothelial fibrinolytic function.

Numerous studies have reported a favorable effect of estrogens on PAI-1 activity and antigen with a concomitant decrease in venous t-PA antigen.^{17,18,25,31,32} The route of administration also determines the effect of HRT on the fibrinolytic system. In this regard, transdermal HRT avoids the first-pass effect of estrogen in the liver and decreases PAI-1 and t-PA antigen to a

lesser extent than oral HRT.¹⁸ Estrogen has been reported to have no effect or to increase³¹ peripheral t-PA activity. The present study is unique in measuring the effect of 17 β -estradiol on basal vascular release of active t-PA and confirms that 17 β -estradiol has a profibrinolytic effect. Although it is important to note that this contrasts with the overall finding of an increased risk of thrombotic events in the WHI study,^{6,7} our subjects were treated with 17 β -estradiol alone rather than with conjugated equine estrogens, did not receive concomitant progesterone and were younger than those enrolled in the WHI study and many prior studies.¹² Thus, these data are consistent with the recent observation that the effects of 17 β -estradiol on vascular endothelial function may depend on age.^{33,34}

Stored t-PA is released from the endothelium both constitutively and in response to activation of G protein-coupled receptors such as the bradykinin B₂ receptor.²⁹ We have reported previously that ACE inhibition enhances basal t-PA antigen release through bradykinin B₂ receptor-dependent pathway²² and that this effect is seen in premenopausal and postmenopausal women but not in men.²³ However, ACE inhibition potentiates bradykinin-stimulated t-PA release to a greater extent in premenopausal women compared with postmenopausal women, suggesting that hormonal status may influence stimulated t-PA release during ACE inhibition.²³ In the present study we again found that ACE inhibition increased basal net t-PA antigen release in postmenopausal women. Interestingly, there was no effect of ACE inhibition on basal release of active t-PA, suggesting that t-PA was rapidly inactivated by PAI-1 or other inhibitors.

Contrary to our hypothesis, however, 1-month treatment with oral 17 β -estradiol did not affect sensitivity to exogenous bradykinin in the presence or absence of ACE inhibition. These data conflict with the findings of a prior study indicating that acute intraarterial administration of 17 β -estradiol increased bradykinin-stimulated t-PA antigen release in postmenopausal women, although forearm venous concentrations of 17 β -estradiol achieved in that study (169.0 \pm 13.6 pg/mL) were higher than those achieved with usual doses of oral 17 β -estradiol.¹⁶ Our data also conflict with observational data from the same study suggesting that bradykinin-stimulated t-PA release was higher in women who took unopposed estrogen therapy. As addressed by the investigators, this prior study was not randomized; in addition, subjects were older than in the current study, and subjects were exposed to estrogen for at least 1 year. On the other hand, data from the present study are compatible with the work of Jern and coworkers who report no effect of acute 17 β -estradiol infusion on methacholine-stimulated forearm blood flow or net t-PA release in postmenopausal diabetic women.³⁵ Taken together with our prior observation that basal and bradykinin-stimulated t-PA release are increased in ACE inhibitor-treated postmenopausal women compared with age-matched men, the data support an estrogen-independent effect of gender on vascular t-PA release during ACE inhibition. In this regard, decreased bradykinin-stimulated t-PA release in men compared with women during ACE inhibition could reflect a reported negative effect of testosterone on bradykinin-stimulated calcium influx,³⁶ a prospect that merits further investigation.

Perspectives

CHD is the leading cause of death in women. ACE inhibitors decrease mortality attributable to CHD in both men and women.³⁷ Previous studies indicate that ACE inhibition increases basal and bradykinin-stimulated t-PA antigen release in pre- and postmenopausal women compared with age-matched men, but enhances bradykinin-stimulated t-PA release to a greater extent in premenopausal women compared with postmenopausal women.²³ The present study confirms that ACE inhibition enhances basal t-PA antigen release in postmenopausal women but suggests that released antigen is rapidly inactivated. The study indicates that 1-month therapy with oral 17 β -estradiol does not alter the effects of ACE inhibition on bradykinin-stimulated t-PA release. However, 17 β -estradiol does increase the basal release of active t-PA

in young postmenopausal women, confirming that decreased PAI-1 antigen concentrations results in enhanced vascular fibrinolytic function. Ongoing clinical trials such as the Kronos Early Estrogen Protection Study (KEEPS, ClinicalTrials.gov Identifier: NCT00154180) and the Early versus Late Intervention Trial with estradiol (ELITE, ClinicalTrials.gov Identifier: NCT0011451) will address whether early versus late estradiol treatment affects thrombotic risk in healthy postmenopausal women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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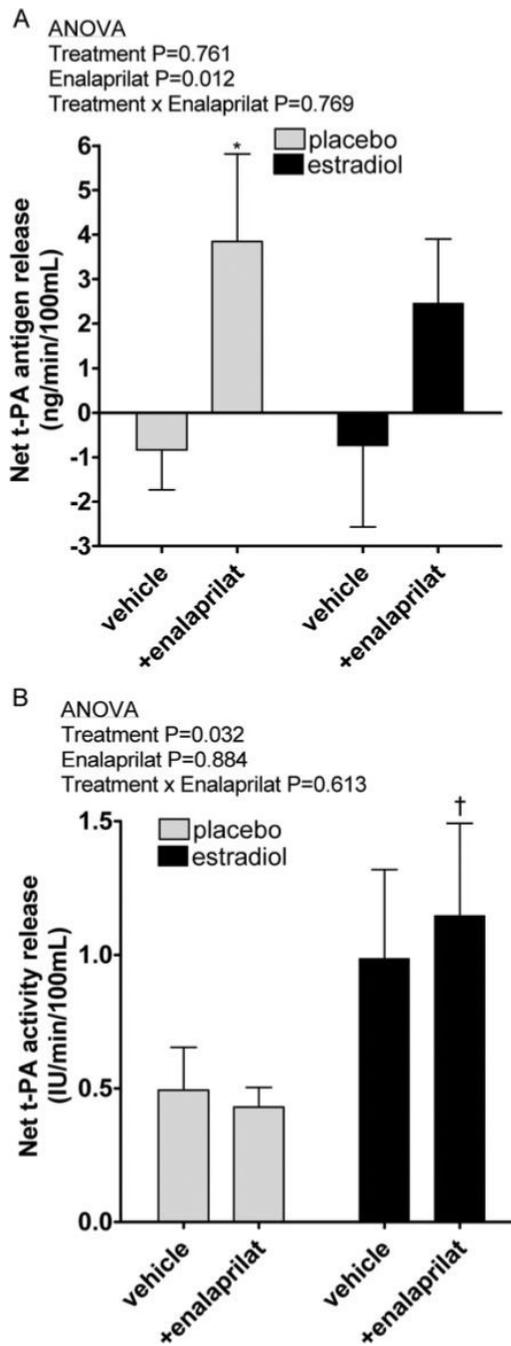


Figure 1. Basal net t-PA antigen release (A) and activity release (B) during placebo and 17 β -estradiol (1 mg/d) treatment, in the presence and absence of intraarterial enalaprilat. * $P=0.039$ vs vehicle, † $P=0.033$ vs placebo, in posthoc analysis.

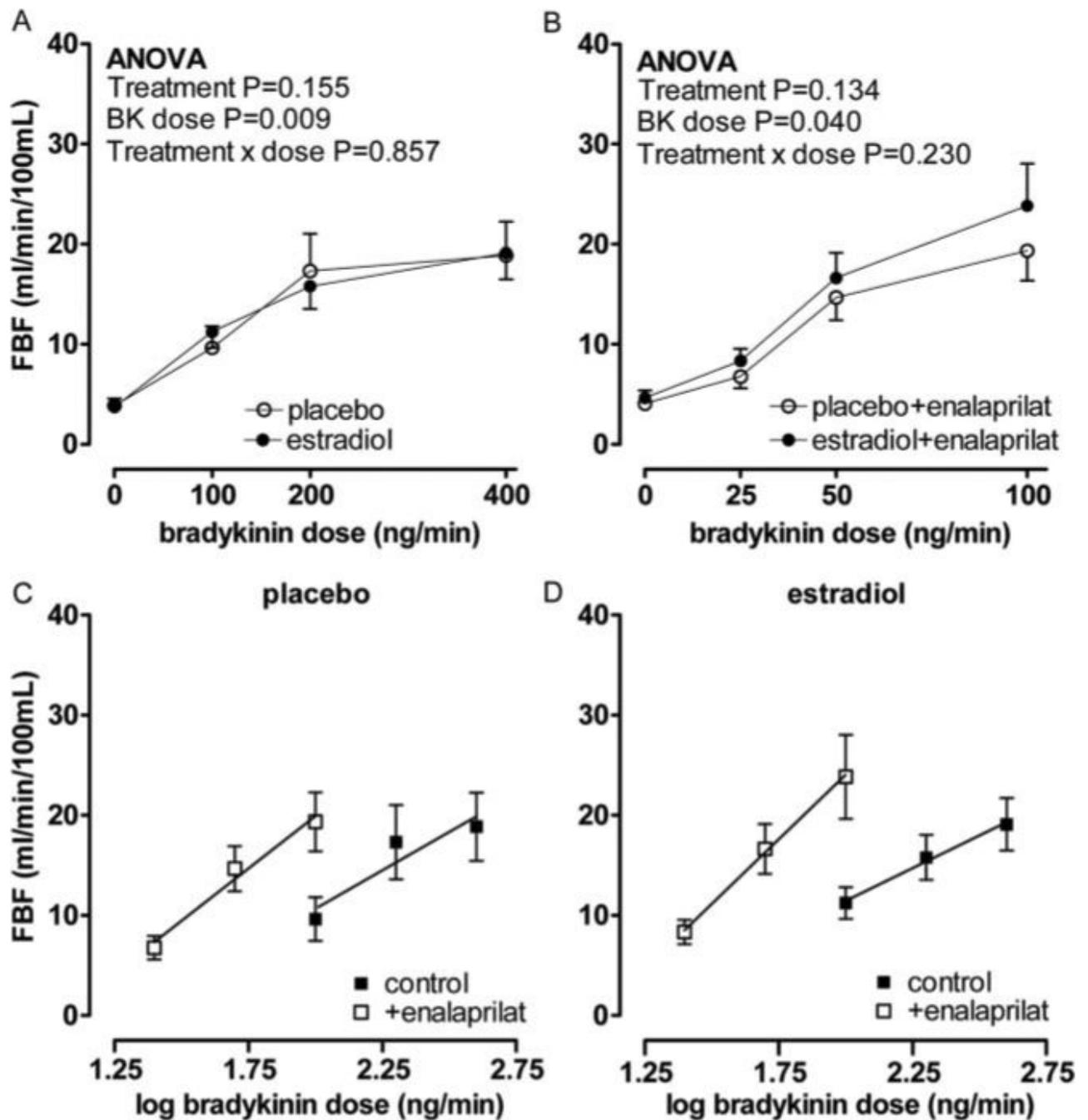


Figure 2.

Forearm blood flow (FBF) response in postmenopausal women to bradykinin (A), bradykinin plus enalaprilat (B) during placebo (○) and 17β -estradiol (1 mg/d) treatment (●). Probability values are for repeated measures ANOVA with pairwise comparisons between placebo and 17β -estradiol. Forearm blood flow versus log bradykinin dose in postmenopausal women in the absence (■) and presence of enalaprilat (□) during placebo (C) and 17β -estradiol treatment (D).

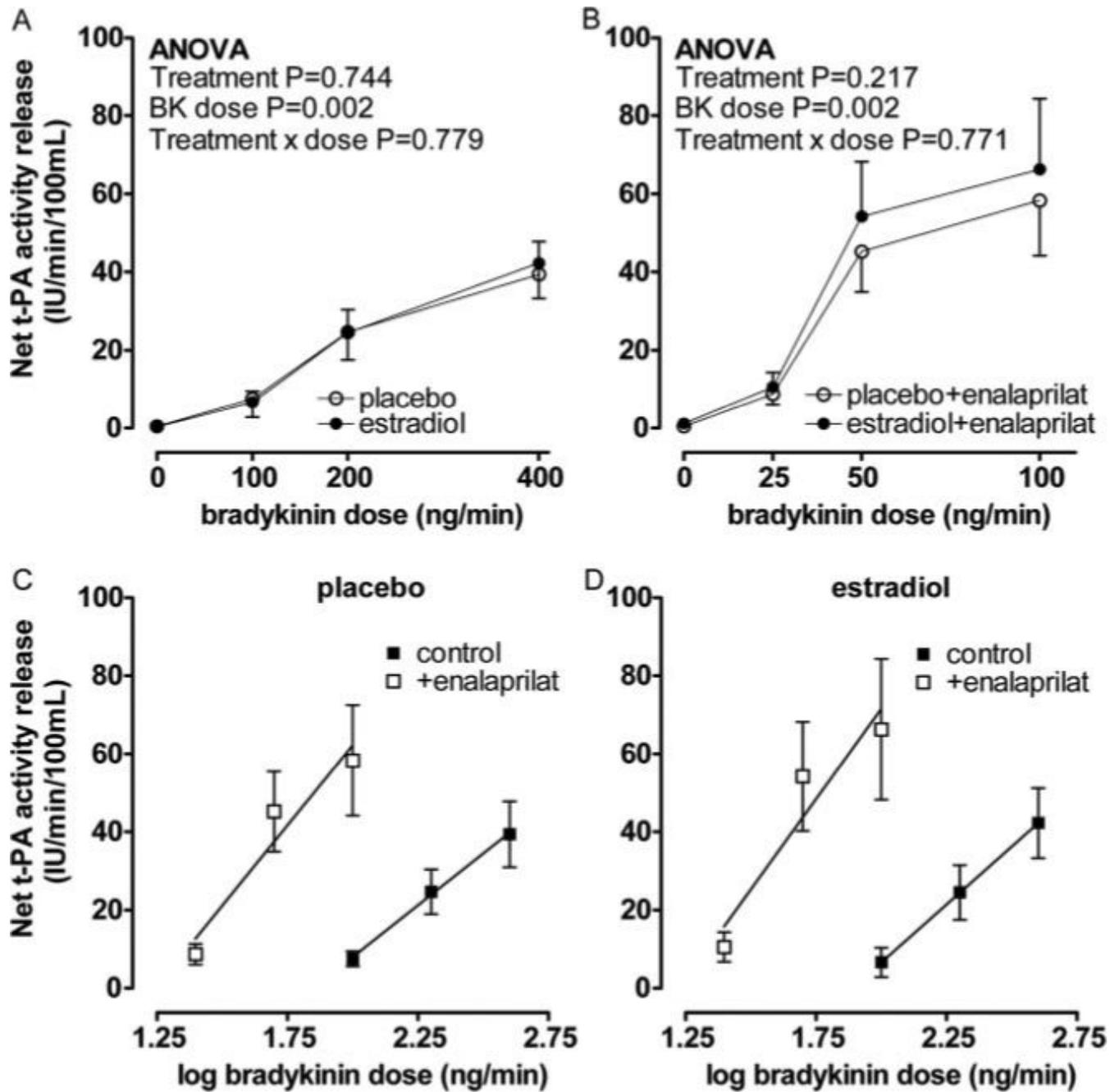


Figure 3. Net release of t-PA activity in response to bradykinin (A), bradykinin plus enalaprilat (B) during placebo (○) and 17β-estradiol treatment (●). Probability values are for repeated measures ANOVA with pairwise comparisons between placebo and 17β-estradiol. Release of t-PA activity versus log bradykinin dose in postmenopausal women in the absence (■) and presence of enalaprilat (□) during placebo (C) and 17β-estradiol treatment (D).

Table

Effect of Treatment on Baseline Characteristics

Parameter	Placebo	17 β -Estradiol	P Value
Systolic blood pressure, mm Hg	112.0 \pm 3.1	110.3 \pm 2.7	0.584
Diastolic blood pressure, mm Hg	69.4 \pm 1.6	68.3 \pm 2.4	0.747
Mean arterial pressure, mm Hg	83.6 \pm 1.7	82.3 \pm 2.3	0.650
Heart rate, bpm	67.9 \pm 2.4	68.9 \pm 2.7	0.492
Baseline FBF, mL/min per 100 mL	3.6 \pm 0.6	4.0 \pm 0.7	0.174
Baseline FVR, mL/min/mm Hg per 100 mL	30.3 \pm 4.9	28.4 \pm 3.3	0.108
Baseline venous PAI-1 antigen, ng/mL	10.4 \pm 2.5	4.4 \pm 1.4	0.001
Baseline venous t-PA antigen, ng/mL	7.5 \pm 1.3	5.5 \pm 0.6	0.022
Baseline venous t-PA activity, IU/mL	0.6 \pm 0.1	0.7 \pm 0.1	0.415

FBF indicates forearm blood flow; FVR, forearm vascular resistance; PAI-1, plasminogen activator inhibitor-1; t-PA, tissue-type plasminogen activator.