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AN EVALUATION OF CONCENTRATION DEPENDENT VASODILATOR EFFECTS OF 17 β-ESTRADIOL USING RAT AORTA

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ABSTRACT

BACKGROUND: *The clinical observation that coronary artery disease is more common in men and postmenopausal women than in premenopausal women has suggested cardioprotective effects of female sex hormones. Estrogen therapy in postmenopausal women has been found to be associated with a decreased risk of heart disease. The mechanisms that mediate this apparent benefit are still unclear. Suggested cardioprotective effects of estrogen include coronary vasodilation. This study was designed to evaluate the concentration dependent vasodilator effects of 17β-estradiol in rat aorta.*

METHODS: *The experimental protocol was divided into two groups. In group I tissue was subjected to serial dilutions of norepinephrine and a standard concentration was selected, producing approximately 75% of maximum vasoconstriction. In group II, tissue was challenged with serial dilutions of 17β-estradiol in the presence of vasoconstriction induced by standard concentration of norepinephrine. All the responses were recorded as rate per 30 seconds on Kymograph machine.*

RESULTS: *In our study 17β-estradiol produced vasodilator effects against the vasoconstrictor effects of norepinephrine. The difference of mean rate increased with increasing concentration of the drug, maximum observed mean rate at the highest concentration was 18.13±0.3 against the lowest mean rate 12.5±0.33. The difference in mean rate in both groups was found to be highly significant ($P<0.001$) at all the concentration levels except at 10^{-8} and 10^{-7} g/ml concentrations.*

CONCLUSION: *These results demonstrate the vasodilator effects of 17b-estradiol, in a concentration-dependent manner.*

KEYWORDS: Estrogen, Vasodilation, Postmenopausal syndrome, Coronary artery Disease.

INTRODUCTION

Coronary artery disease is one of the most common and costly diseases. Coronary vasospasm and subsequent occlusion are frequently associated with increased cardiovascular risk and may lead to myocardial infarction and death[1]. Cardiovascular disease is the leading killer of postmenopausal women in United States and in most developed countries[2].

Most of the common forms of the cardiovascular disease such as atherosclerosis are caused by functional and structural changes in the blood vessel wall. These changes include abnormal vasoconstriction, enhanced interaction of blood cells with blood vessel wall, activation of coagulation mechanisms, and migration and proliferation of vascular smooth muscle cells[3].

Women appear to be protected until the menopause, from the development of the coronary artery disease. This protective effect seems to be due to the beneficial effect of ovarian hormones, in particular 17β-estradiol, since estrogen replacement therapy reduces the incidence of coronary artery disease and progression of coronary artery lesions in postmenopausal women. Estrogens elicit a myriad of biological responses, and it has been appreciated since the early 1960s that these actions are receptor mediated[4]. A number of potential mechanisms for the protective effect of estrogens have been proposed. One of the main beneficial actions of estrogen may be to decrease low density lipoprotein (LDL) and increase high density lipoprotein (HDL) cholesterol[5]. However, these lipid changes have been calculated to be insufficient to explain the almost 50% reduction in events observed with treatment. Thus additional mechanisms of estrogen benefits must be considered[6].

Epidemiological and observational studies support a vasculoprotective effect of estrogens[7]. Estrogen may exert an independent cardioprotective effect through direct action on coronary dynamics[8]. Estrogen has been reported to dilate both arterial and venous smooth muscles[9]. A number of studies have shown that estrogens modulate vasomotor responses after both acute application and in vivo short- or long-term treatment[10,11]. Such effects seem to be mediated by specific estrogen receptors (ERs) that are located on the plasma membrane as well as intracellularly[12]. So far, two ER subtypes have been described: ERá and ERâ. Both subtypes are found in vascular smooth muscle[13,14] and human endothelial cells[15]. Recently published reviews on ERâ have examined estrogen actions in general and lessons learnt from in vitro studies[16,17].

This study was designed to evaluate the concentration dependent vasodilator effects of 17β-estradiol.

MATERIALS AND METHODS

This study was conducted at the Department of Pharmacology and Therapeutics, BMSI, JPMC, Karachi.

The drugs used were 17β-estradiol and norepinephrine. Ringer's solution was used for the nutrition purpose. Adult male Albino rats were divided into two groups of eight animals each. The animals were killed by a blow on the head with hammer. The abdomen was opened by a midline incision, aorta was located and other unnecessary tissues and visceras were parted. The aorta was cannulated and perfused with Ringer's solution, and the preparation was mounted on the myograph board. The perfusate coming out of cut vessels was collected in the petri dish. Falling of drops over the sensor of drop recording unit was recorded on the recording paper placed over drum of Kymograph machine as rate per 30 seconds.

In group I, tissue was subjected to serial dilutions of norepinephrine and responses were recorded. The standard concentration was selected which produced approximately 75% of maximum vasoconstriction.

In group II, tissue was challenged with serial dilutions of 17b-estradiol in the presence of standard concentration of norepinephrine.

Statistical analysis was performed by using the Sign Test and Mann Whitney's Rank Sum Test.

RESULTS

3.1. Response of Rat Aorta after Administration of Norepinephrine (Group I)

Table 1

RESPONSE OF AORTA OF RAT AFTER ADMINISTRATION OF NOREPINEPHRINE

(All values are expressed in Mean±SEM)

DC	BL (Rate per 30 sec)	DR1 (Rate per 30 sec)	% Diff.
10^{-12}	19.75±0.45	19.50±0.53	1.26
10^{-11}	19.75±0.45	18.50±0.60	6.32
10^{-10}	19.75±0.45	17.63±0.46	10.23
10^{-9}	19.75±0.45	17.13±0.44	13.26
10^{-8}	19.75±0.45	16.00±0.46	18.98
10^{-7}	19.75±0.45	14.75±0.37	25.31
10^{-6}	19.75±0.45	13.75±0.31	30.37
10^{-5}	19.75±0.45	12.75±0.31	35.44
10^{-4}	19.75±0.45	11.88±0.23	39.84
10^{-3}	19.75±0.45	10.38±0.18	47.44

DC = Drug concentration (g/ml)
BL = Baseline
DR1 = Drug 1 (Norepinephrine)
% Diff. = Percentage difference

Table 2

RESPONSE OF AORTA OF RAT AFTER ADMINISTRATION OF 17B-ESTRADIOL IN THE PRESENCE OF STANDARD CONCENTRATION OF NOREPINEPHRINE

(All values are expressed in Mean±SEM)

DC	DR1 (Rate per 30 sec)	DR2 (Rate per 30 sec)	% Diff.
10^{-12}	12.25±0.25	12.38±0.18	1.06
10^{-11}	12.25±0.25	13.25±0.25	8.16
10^{-10}	12.25±0.25	14.25±0.25	16.32
10^{-9}	12.25±0.25	14.88±0.23	21.46
10^{-8}	12.25±0.25	15.63±0.32	27.59
10^{-7}	12.25±0.25	16.38±0.32	33.71
10^{-6}	12.25±0.25	17.00±0.27	38.77
10^{-5}	12.25±0.25	17.88±0.23	45.95
10^{-4}	12.25±0.25	18.88±0.30	54.12
10^{-3}	12.25±0.25	19.75±0.37	61.22

DC = Drug concentration (g/ml)
DR1 = Drug 1 (Norepinephrine)
DR2 = Drug 2 (17b-Estradiol)
% Diff. = Percentage difference

The difference between responses at baseline and drug expressed in percentage showed that norepinephrine gradually decreased the mean rate from 19.75 to 10.38 with increasing concentrations. The mean values with percentage difference are shown in table 1 and graphically plotted in figure 1. From these observations the concentration of 10^{-5} g/ml was selected as standard concentration, which produced the optimal vasoconstriction. Statistically concentration to concentration difference in mean of rate was found to be significant at all the levels except baseline to 10^{-12} and 10^{-10} to 10^{-9} g/ml concentration levels ($P<0.05$).

3.2. Response of Rat Aorta after

Administration of 17b-Estradiol in the Presence of Standard Concentration of Norepinephrine (Group II)

The mean change of rate from baseline to standard concentration of norepinephrine was found to be 8.88 (21.13 to 12.25) per 30 seconds and the mean change of rate was increased gradually from 12.38 to 19.75 (7.37) per 30 seconds with increasing concentration of 17b-Estradiol showing the magnitude of vasodilator action of drug. The mean values and percentage differences are shown in table 2 and graphically plotted in figure 2.

Statistically the differences at all levels were found to be significant except at the concentration levels of 10^{-10} to 10^{-9} and 10^{-7} to 10^{-6} g/ml ($P<0.05$).

The mean rate was compared at each level of concentration between Group I and Group II to evaluate the difference between vasoconstrictor effects of norepinephrine and vasodilator effects of 17β-estradiol as shown in table 3 and graphically plotted in figure 3. From these observations the results were found to be non-significant at the levels of 10^{-8} and 10^{-7} g/ml concentrations, remaining all concentration levels resulted in being statistically highly significant ($P<0.001$).

Table 3

COMPARISON OF RATE AT DIFFERENT LEVELS OF CONCENTRATIONS OF NOREPINEPHRINE AND 17B-ESTRADIOL BETWEEN GROUP I AND GROUP II

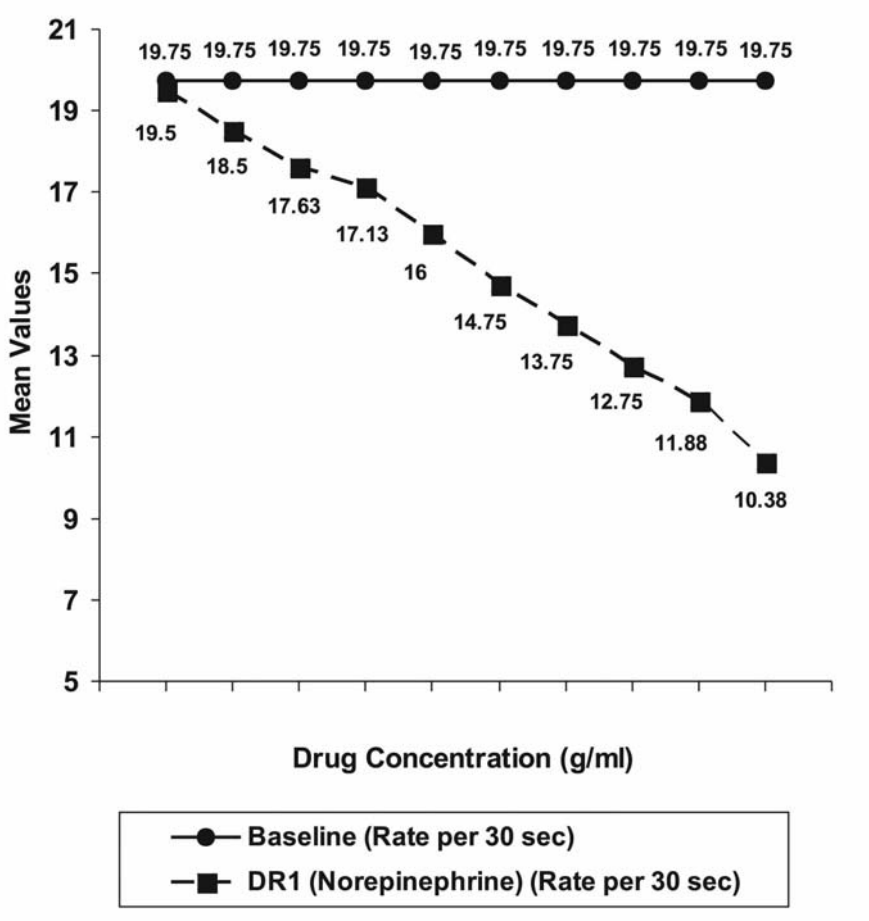
(All values are expressed in Mean±SEM)

DC	DR1 (Rate per 30 sec)	DR2 (Rate per 30 sec)	% Diff.
10^{-12}	19.50±0.53	12.38±0.18	36.51
10^{-11}	18.50±0.60	13.25±0.25	28.37
10^{-10}	17.63±0.46	14.25±0.25	19.17
10^{-9}	17.13±0.44	14.88±0.23	13.13
10^{-8}	16.00±0.46	15.63±0.32	2.31
10^{-7}	14.75±0.37	16.38±0.32	11.05
10^{-6}	13.75±0.31	17.00±0.27	23.63
10^{-5}	12.75±0.31	17.88±0.23	40.23
10^{-4}	11.88±0.23	18.88±0.30	58.92
10^{-3}	10.38±0.18	19.75±0.37	90.26

DC = Drug concentration (g/ml)
DR1 = Drug 1 (Norepinephrine)
DR2 = Drug 2 (17b-Estradiol)
% Diff. = Percentage difference

Figure 1

RESPONSE OF AORTA OF RAT AFTER ADMINISTRATION OF NOREPINEPHRINE



4. DISCUSSION

Gender specific differences in the incidence of cardiovascular disease were recognized half a century ago. It is now well established that while the risk of heart disease in men increases constantly with age, premenopausal women have a significantly lower risk, which however, increases rapidly after menopause to levels comparable to that of their male counterparts. In fact, in postmenopausal women receiving estrogen substitution, the cardiovascular mortality is 30 – 50% less than that found in their untreated female counterparts. Based on these epidemiological data, it has been suggested that estrogen protects against cardiovascular disease. The research related to the mechanism of estrogen actions has indicated that the cardioprotective effects of estrogen are mediated by an antiatherogenic action on the lipid profile and by direct effects on arterial wall[18]. Estrogen has been reported to directly induce vasodilation in the coronary circulation[19]. The objective of present in vitro study was to evaluate the concentration dependent vasodilator effect of 17β-estradiol.

In our study we observed the vasodilator effects of 17β-estradiol in aorta of rat in Group II after induction of vasoconstriction by standard concentration of norepinephrine selected in Group I. Results were found to be significant for 17b-estradiol, as being

vasodilator agent and the vasodilator activity being increased with increasing concentration of the drug.

Our results are consistent with Gisclard V et al (1988)[20], who observed the vasodilatation caused by 17b-estradiol in ovarectomized female New Zealand rabbits. Williams JK et al (1992)[21] determined the effects of short term administration of estrogen on endothelium-mediated dilation in the coronary arteries of 13 ovarectomized Cynomolgus monkeys. The arteries constricted in response to infusion of acetylcholine before estrogen treatment but dilated 20 minutes after intravenous injections of ethinyl estradiol ($P<0.5$). Vascular responses of coronary arteries, both before and after administration of estrogen, were not associated with variations in plasma lipid concentrations, blood pressure, heart rate or plaque size. Collins P et al (1995)[22], reported estrogen to show a direct relaxing effect on the vascular smooth muscles of rabbit coronary arteries. Concentrations of estrogen used in those experiments were above physiological concentrations (10^{-6} and 10^{-5} mol/L).

Cockell A and Poston L (1997)[23] observed that 17b-estradiol stimulated flow induced relaxation in the resistance vasculature of young female rats. Kakucs R et al (2001)[24] demonstrated the direct, vasorelaxant effect of estradiol on a peripheral small artery in ovarectomized female rats, estrogen replacement induced a morphological dilation.

Results obtained from these studies on different animal models support our results of 17b-estradiol to have vasodilator effect, which was found increasing with increasing concentration.

CONCLUSION

Our observations demonstrate the vasodilator effects of 17b-estradiol in a concentration-dependent manner. These vasodilator effects of 17β-estradiol may support a possible role of hormonal replacement therapy in protection against coronary artery disease in post-menopausal women.

REFERENCES

1. Crews JK and Khalil RA, Antagonist effects of 17 b Estradiol, progestrone and testosterone on Ca^{2+} entry mechanisms of coronary vasoconstriction. Anterioscler Thromb Vasc Biol 1999; 19:1034-1040.
2. Nabel EG. Coronary heart disease in women. An ounce of prevention. N Engl J Med 2000; 343:572-574.
3. Luscher TF. The endothelium and cardiovascular disease. A complex relation. N Engl J Med 1994; 330:1081-1083.
4. Harris HA. Estrogen Receptor-β: Recent

Figure 2

RESPONSE OF AORTA OF RAT AFTER ADMINISTRATION OF 17β-ESTRADIOL IN THE PRESENCE OF STANDARD CONCENTRATION OF NOREPINEPHRINE

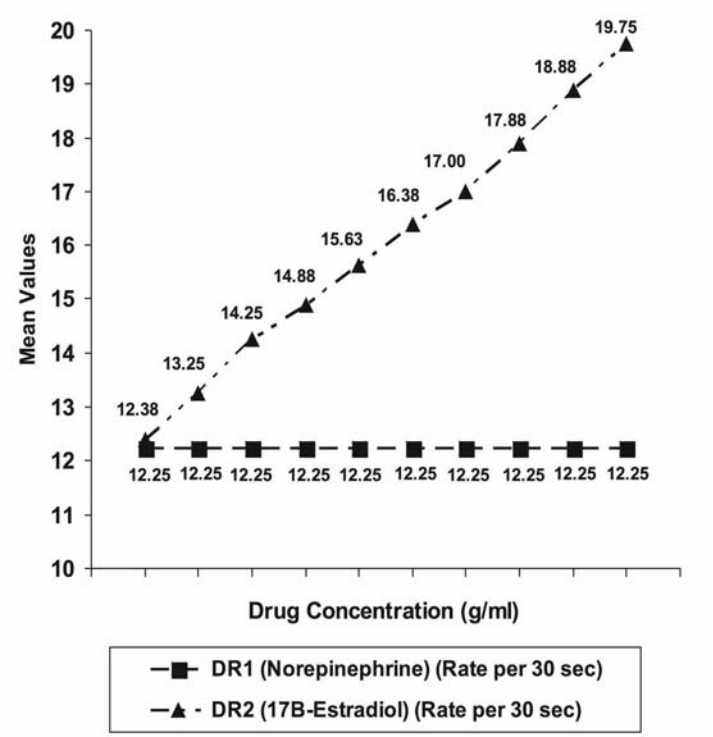
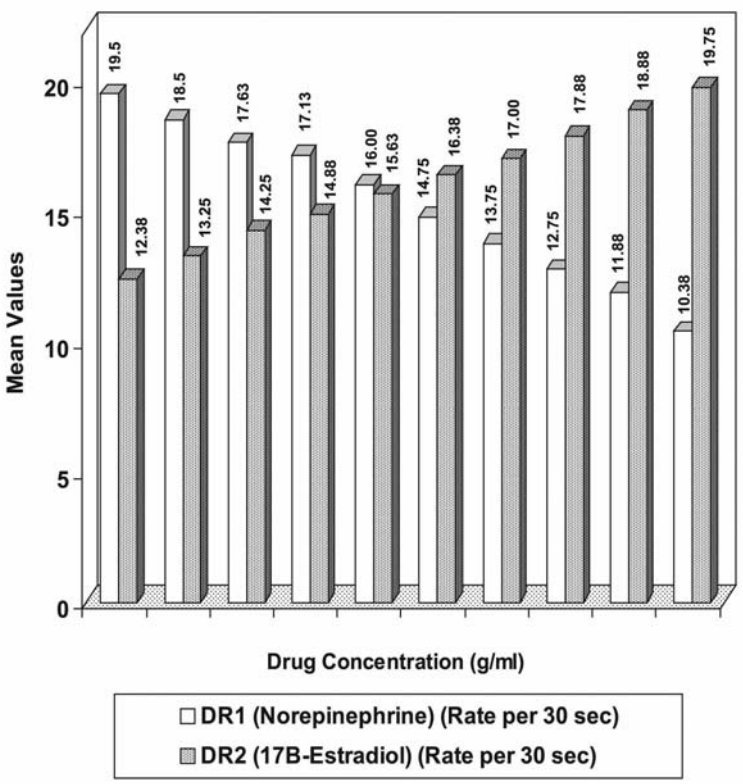


Figure 3

COMPARISON OF RATE AT DIFFERENT LEVELS OF CONCENTRATIONS OF NOREPINEPHRINE AND 17β-ESTRADIOL BETWEEN GROUP I AND GROUP II



Lessons from in Vivo Studies. Mol, Endocrinol 2007; 21:1–13.
5. Belfort MA, Saade GR, Wen TS et al. The direct action of 17b estradiol in isolated omental artery from non-pregnant and pregnant women is related to calcium antagonism. Am J Obstet Gynecol 1996; 175:1163-1172.
6. Gilligan DM, Badar DM, Panza JA et al. Acute vascular effects of estrogen in postmenopausal women. Circulation 1994; 90:786-791.
7. Brouchet L, Krust A, Dupent S et al; Estradiol accelerates reendothelialization in mouse carotid artery through estrogen receptor a but not estrogen receptor b. Circulation 2001; 103:423-428.
8. Bing RJ and Conforto A. Reversal of acetylcholine effect on atherosclerotic coronary arteries by estrogen: Pharmacologic phenomenon of clinical importance. J Am Coll Cardiol 1992; 20:458-459.
9. Belfort MA, Saade GR, Suresh M et al. Effects of estradiol 17b and progesterone on isolated human omental artery from postmenopausal non-pregnant women and from normatensive and preeclamptic pregnant women. Am J Obstet Gynecol 1996; 174:246-253.
10. Mendelsohn ME and Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med 1999; 340:1801–1811.
11. Cignarella A, Paoletti R, and Puglisi L. Direct effects of estrogen on the vessel wall. Med Res Rev 2001; 21:171–184.
12. Bolego C, Cignarella A, Sanvito P, et al. The Acute Estrogenic Dilation of Rat Aorta Is Mediated Solely by Selective Estrogen Receptor-_ Agonists and Is Abolished by Estrogen Deprivation. JPET 2005; 313:1203–1208.
13. Hodges YK, Tung L, Yan XD, et al. Estrogen receptors á and â: prevalence of estrogen receptor â mRNA in human vascular smooth muscle and transcriptional effects. Circulation 2000; 101:1792–1798.
14. Maggi A, Cignarella A, Brusadelli A, et al. Diabetes undermines estrogen control of inducible nitric oxide synthase function in rat aortic smooth muscle cells through overexpression of estrogen receptor-â. Circulation 2003; 108:211–217.
15. Caulin-Glaser T, Watson CA, Pardi R, and Bender JR (1996) Effects of 17 β –estradiol on cytokine-induced endothelial cell adhesion molecule expression. J Clin Investig 1996; 98:36–42.
16. Koehler KF, Helguero LA, Haldosen LA, Warner M, Gustafsson JA. Reflections on the discovery and significance of estrogen receptor β. Endocr inor Rev 2005; 26:465–478.
17. Hewitt SC, Harrell JC, Korach KS. Lessons in estrogen biology from knockout and transgenic animals. Annu Rev Physiol 2005; 67:285–308.
18. Javonovic A and Javonovic S. Estrogen and vascular system: More questions for future. Cardiovasc Res 1992; 42:9-11.
19. Sudhir K, Chou TM and Mullen WL. Mechanisms of estrogen induced vasodilation: In vitro studies in canine coronary conductance and resistance arteries. J Am Coll Cardiol 1995; 26:807-814.
20. Gisclard V, Miller VM and Vanhoutte PM. Effects of 17b estradiol on endothelium-dependent responses in the rabbit. J Pharmacol Exp Ther 1988; 244:19-22.
21. Williams JK, Honore EK, Washburn SA et al. Effects of hormone replacement therapy on reactivity of atherosclerotic coronary arteries in Cynomolgus monkeys. J Am Coll Cardiol 1994; 24:1757-1761.
22. Collins P, Rosano GMC, Sarrel PM et al. 17b estradiol attenuates acetylecholine-induced coronary arterial constriction in women but not men with coronary heart disease. Circulation 1995; 92:24-30.
23. Cockel AP and Poston L. 17b estradiol stimulates flow induced vasodilation in isolated mesenteric arteries from prepubertal female rats. Am J Obstet Gynecol 1997; 177:1432-1438.
24. Kakucs R, Varbiro S, Nadasy GL, Monos E, Szekacs B. Acute nongenomic vasodilatory action of Estradiol is attenuated by chronic Estradiol treatment. Exp Biol Med 2001; 226:538-542.