ROLE OF CALCIUM CHANNEL ANTAGONISM IN VASODILATOR EFFECTS OF 17β-ESTRADIOL

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Background: Cardiovascular events are less prevalent in premenopausal women and women receiving oestrogen replacement than in postmenopausal women or men. The mechanisms that mediate this apparent benefit are still unclear. Suggested mechanisms include coronary vasodilatation. The vasodilator activity is mainly suggested to be mediated through action on calcium ion channel antagonism and nitric oxide production from the vascular endothelium. This study was aimed to evaluate the extent of role of calcium channel blockade underlying the vasodilator action of the 17β-estradiol. Materials and Methods: 32 male albino rats were divided into three groups. In group I, tissue was challenged with serial dilutions of norepinephrine and standard concentration was selected producing approximately 75% of maximum vasoconstriction. In group II, tissue was challenged with verapamil and standard concentration was selected in the presence of norepinephrine mediated vasoconstriction, and in group III, tissue was challenged with serial dilution of 17β-estradiol after pretreatment with verapamil the presence of norepinephrine mediated vasoconstriction. Results: Results obtained showed that vasodilator activity of both the drugs was almost similar up to 10^{-7} gm/ml concentration. At higher concentrations, 17β-estradiol showed greater vasodilator response suggesting the possibility of presence of other mechanisms independent of calcium channel antagonism in addition to calcium channel blockade. Conclusion: Our observations suggest that vasodilator activity of 17-β-estradiol is not mediated through calcium channel antagonism only. It suggests the possibility of other mechanism(s) also, underlying these cardioprotective effects in post menopausal women.

Keywords: Vasodilation, Calcium channel antagonism, Postmenopausal syndrome.

INTRODUCTION

Cardiovascular disease is No. 1 killer of women in United States and worldwide and marked disparities in cardiovascular health exists between men and among groups of women.[1] There is bias in the prevalence of cardiovascular morbidity and mortality until menopause; thereafter this difference is lost.[2] Endogenous estrogens have been implicated in protection from cardiovascular disease in premenopausal women, and accordingly lack of oestrogen is thought to be in part responsible for accelerated development of atherosclerosis in men and postmenopausal women.[3] Over the last four decades, epidemiological and case controlled studies, showing favourable changes in cardiovascular risk factors with oestrogen supplementation, have suggested that oestrogen replacement therapy in post menopausal women might be beneficial in terms of prevention of adverse cardiovascular events[4] Hormone therapy is used to treat undesirable symptoms associated with menopause and is being explored for diseases such as Alzheimer's disease and stroke.[5] The vascular wall is clearly one of the target organs of oestrogen.[6] Oestrogen has been suggested to modulate vascular physiology and function from a variety of studies in cellular, animal and human models.[7] The vascular effects of oestrogen are complex and occur at multiple levels.[8] The finding that 17β-estradiol (17β-E_2) administration results in rapid vasodilatation, attributed to a plasma membrane ER, has provoked interest in the potential role of nongenomic activation of the ER in vascular homeostasis. Nitric Oxide (NO) has been implicated, but its role remains controversial, since both NO dependent and independent dilation has been reported in ex vivo studies using arteries.[9] Oestrogen may also induce vasodilatation through potential effects on catecholamine release or inhibition of extra neuronal uptake norepinephrine (uptake 2) or through calcium channel antagonism.[10] Han *et al* demonstrated that 17β-estradiol inhibited the Ca^{2+} influx stimulated by the receptor agonist U46619 and high K^{+} depolarization in isolated porcine coronary arteries without changing the Ca^{2+} sensitivity of contractile elements. These effects were similar to those observed with Ca^{2+} channel blockers. 17β-estradiol also inhibited the Ca^{2+} release induced by agonist U46619 at higher concentration.[11] Javonovic *et al* also referred endothelium-independent oestrogen induced vasorelaxation was usually ascribed to the

blockade of Ca^{2+} channels and inhibition of Ca^{2+} influx.[12]

Present in vitro study was aimed to evaluate the extent of role of calcium channel blockade underlying the vasodilator activity of the 17β-estradiol.

MATERIAL AND METHODS

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

The drugs used were 17β-estradiol, norepinephrine and verapamil. Ringer's solution was used in this study for the nutrition purpose. Adult male Albino rats were divided into three 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 a 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 response. In group II, tissue was subjected to serial dilutions of verapamil and responses were recorded, the standard concentration was selected which produced approximately 75% of maximum response in the presence of norepinephrine mediated vasoconstriction. In group III, tissue was challenged with serial dilutions of 17β-estradiol in the presence of standard concentration of norepinephrine after pretreatment with verapamil. Statistical analysis was performed by using the Sign Test and Mann Whitney's Rank Sum Test.

RESULTS

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

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 ($p<0.05$).

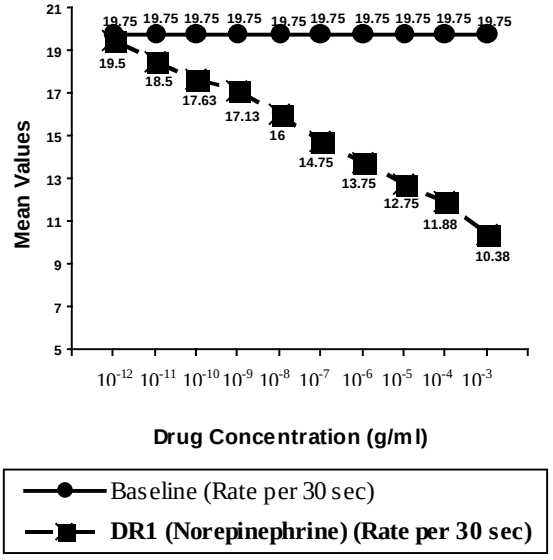

Figure 1: Response of aorta of rat after administration of norepinephrine (Group I)

Response of Rat Aorta after Administration of Verapamil in the Presence of Standard Concentration of Norepinephrine (Group II)

The difference between responses after administration of standard concentration of norepinephrine and various concentrations of verapamil showed that verapamil increased the mean rate from 12.50 to 18.13 per 30 seconds (Table-2). Among the various concentrations of verapamil 10^{-5} g/ml concentration was selected as standard concentration, which produced optimal vasodilation. Statistically concentration to concentration level results were found to be non-significant.

Response of Rat Aorta after Administration of 17β-Estradiol in the Presence of Standard Concentration of Norepinephrine after pre treatment with Standard Concentration of Verapamil (Group III)

The difference between responses after administration standard concentrations of norepinephrine and verapamil increased from 10.88 to 16.00 at different levels when applied for evaluating the potential effects of 17β-estradiol, while the mean change of rate after administration of various concentration of 17β-estradiol increased from 16.00 to 18.87 (table 3). Statistically the difference between verapamil to 10^{-5} g/ml, 10^{-4} g/ml and 10^{-3} g/ml concentrations of 17β-estradiol were found significant.

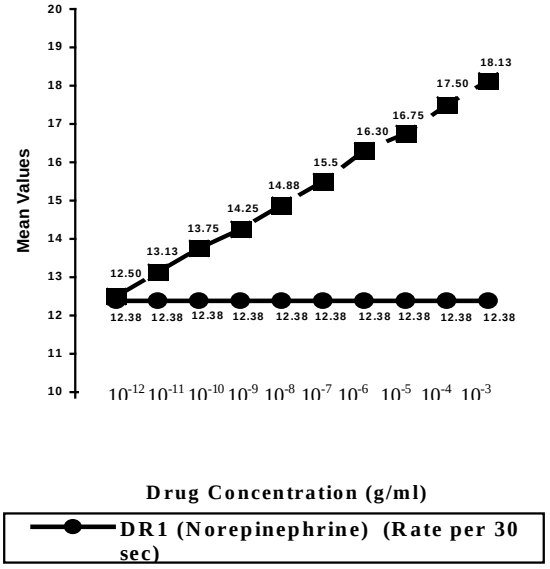

Figure-2: Response of aorta of rat after administration of verapamil in the presence of standard concentration of norepinephrine (Group II)

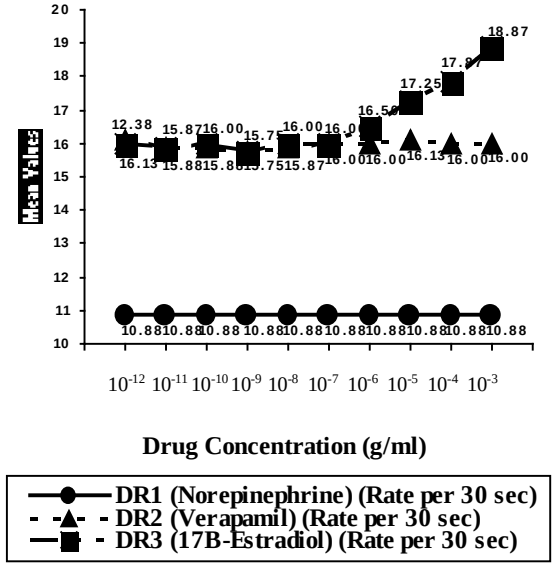

Figure-3: Response of aorta of rat after administration of verapamil (standard concentration) and 17β-estradiol in the presence of standard concentration of norepinephrine (Group III)

Table-1: Response of aorta of rat after administration of norepinephrine (Group I) (Mean±SEM)

Drug concentration (g/ml)	Baseline (Rate per 30 sec)	Drug 1 (Norepinephrine) (Rate per 30 sec)	Percentage difference
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

*Standard concentration

Table-2: Response of aorta of rat after administration of verapamil in the presence of standard concentration of norepinephrine (Group II) (Mean±SEM)

Drug concentration (g/ml)	Drug 1 (Norepinephrine) (Rate per 30 sec)	Drug 2 (Verapamil) (Rate per 30 sec)	Percentage difference
10^{-12}	12.38±0.26	12.50±0.33	0.96
10^{-11}	12.38±0.26	13.13±0.35	6.05
10^{-10}	12.38±0.26	13.75±0.31	11.06
10^{-9}	12.38±0.26	14.25±0.25	15.10
10^{-8}	12.38±0.26	14.88±0.35	20.19
10^{-7}	12.38±0.26	15.50±0.27	25.20
10^{-6}	12.38±0.26	16.30±0.30	31.66
*10^{-5}	12.38±0.26	16.75±0.25	35.29
10^{-4}	12.38±0.26	17.50±0.33	41.35
10^{-3}	12.38±0.26	18.13±0.30	46.44

*Standard concentration

Table-3: Response of aorta of rat after administration of verapamil (standard concentration) and 17β-estradiol in the presence of standard concentration of norepinephrine (group-III) (Mean±SEM)

Drug concentration (g/ml)	Drug 1 (Norepinephrine) (Rate per 30 sec)	Drug 2 (Verapamil) (Rate per 30 sec)	Drug 3 (17β-Estradiol) (Rate per 30 sec)	Percentage difference
SC	10.88±0.23	16.13±0.23	-	48.25
10^{-12}	10.88±0.23	-	16.00±0.19	47.05
SC	10.88±0.23	15.88±0.23	-	45.95
10^{-11}	10.88±0.23	-	15.87±0.23	45.86
SC	10.88±0.23	15.88±0.30	-	45.95
10^{-10}	10.88±0.23	-	16.00±0.19	47.05
SC	10.88±0.23	15.75±0.31	-	44.76
10^{-9}	10.88±0.23	-	15.75±0.31	44.76
SC	10.88±0.23	15.87±0.23	-	45.86
10^{-8}	10.88±0.23	-	16.00±0.19	47.25
SC	10.88±0.23	16.00±0.27	-	47.05
10^{-7}	10.88±0.23	-	16.00±0.19	47.05
SC	10.88±0.23	16.00±0.19	-	47.05
10^{-6}	10.88±0.23	-	16.50±0.19	51.65
SC	10.88±0.23	16.13±0.12	-	48.25
10^{-5}	10.88±0.23	-	17.25±0.16	58.54
SC	10.88±0.23	16.00±0.19	-	47.05
10^{-4}	10.88±0.23	-	17.87±0.23	64.24
SC	10.88±0.23	16.00±0.19	-	47.05
10^{-3}	10.88±0.23	-	18.87±0.23	73.43

SC= Standard concentration

DISCUSSION

Animal and human studies have demonstrated a protective effect of oestrogen on the coronary circulation. In addition to its beneficial effects on lipids, oestrogen has been reported to directly induce vasodilation in the coronary circulation.[10] Oestrogen can cause short-term vasodilation by both endothelium-dependent and endothelium-independent pathways. Two mechanisms for the rapid vasodilatory effects of oestrogen have been explored in some depth; effects on ion channel function and effects on nitric oxide.

In vascular smooth muscle cells, ion channels direct the flow of potassium, sodium and calcium ions into and out of the cell, determining the electrical potential at rest and the contractile state of the smooth muscle. At supra physiologic concentrations, oestrogen inhibits the influx of extra cellular calcium into vascular smooth muscle cells by an effect on cell membrane or L-type calcium channels. However, the high concentrations of oestrogen required and lack of specificity of oestrogen derivatives for its effect on calcium channels suggest that it is largely a pharmacologic phenomenon. At physiologic concentrations, oestrogen stimulates the opening of calcium-activated potassium channels through a nitric oxide and cyclic guanosine mono phosphate dependent pathway, thus relaxing smooth muscle and promoting vasodilation.[13]

Belfort MA *et al*[14] found that 17β-estradiol inhibits norepinephrine induced contraction in human omental arteries. The inhibition of tension developed after exposure to potassium chloride, norepinephrine and calcium ions was caused by a calcium blocking action. Crews JK *et al*[15] in their study on pig model suggested that sex hormone inhibit Ca^{2+} entry into coronary smooth muscles and oestrogen mainly inhibits Ca^{2+} entry through voltage gated Ca^{2+} channels.

In the present study we investigated the extent of role of calcium channel blockade on the vasodilator activity of the 17β-estradiol. For this purpose in group III we challenged the tissue with serial dilutions of 17β-estradiol after pre-treatment with standard concentration of verapamil selected in group II in the presence of vasoconstriction induced by standard concentration of norepinephrine selected in group I. Our findings demonstrated that the results were almost similar up to concentration of 10^{-7} g/ml for both the drugs, beyond that concentration the results were significantly different in terms of greater vasodilator activity of 17β-estradiol suggesting the possibility of 17β-estradiol to have some other

possible mechanism(s) underlying its vasodilator response besides calcium channel antagonism.

CONCLUSION

In the present in vitro study model, we observed the vasodilator response of 17β-estradiol in relation to extent of role of calcium channel antagonism. We observed the vasodilator activity of the drug to be almost similar at lower concentrations, while at higher concentrations 17β-estradiol shows greater response leaving the possibility to have some other mechanism(s) also besides calcium channel antagonism.

Our observations suggest the need of further research in this regard to evaluate other possible mechanism(s) underlying these beneficial effects.

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