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Notes/Citation Information
Published in *The West Virginia Medical Journal*, v. 96, p. 617-621.

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Estrogen-induced relaxation in bovine coronary arteries in vitro: Evidence for a new mechanism

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Abstract

Numerous studies have shown estrogen to be vasoactive in various circulations. Our objective was to determine the effect of estrogen on isolated bovine coronary arteries and the possible mechanism. Bovine coronary arteries, precontracted with thromboxane mimetic U_{46619} were given doses (0.01-30μM) of 17β-estradiol in the presence and absence of endothelium and these inhibitors: 10μM indomethacin (cyclooxygenase inhibitor), 10μM methylene blue (inhibits soluble guanylate cyclase), 100μM nitro-L-arginine (inhibits nitric oxide synthesis), 100μM isobutylmethylxanthine (phosphodiesterase inhibitor) and 30μM mifepristone (Ru_{38486} steroid receptor antagonist). Our results indicated that, estrogen, in the highest concentration used (30μM), elicited an acute dose-dependent relaxation of bovine coronary arteries from 4%-68% (n=15). No major difference in relaxation was observed between coronary arteries with or without endothelium, indicating that the mechanism was endothelium-independent. Indomethacin, nitro-L-arginine and methylene blue did not alter this relaxation, suggesting that relaxant prostaglandins, l-arginine products and cGMP are not involved (n=11-16), isobutylmethylxanthine enhanced relaxation from 20%-40% (n=15 $p < 0.01$), suggests a role for cAMP. Furthermore, mifepristone reduced the relaxation by more than 50% (n=15 $p<0.05$) consistent with the role for estrogen receptors. Based on our study, estrogen causes a dose-dependent relaxation of bovine coronary arteries that does not appear to utilize endothelium, prostaglandins, cGMP or arginine products, but may involve cAMP and estrogen receptors. This study may help justify treating myocardial ischemia with estrogen.

Introduction

Although the cardioprotective effect of estrogen is well recognized (1), mechanisms by which this steroid hormone provides its effect are not fully understood. The cardiovascular protective action of estrogen is reportedly mediated by effects on lipoprotein metabolism, hemostatic factors and by the direct effect on the vessel wall.

One characteristic of estrogen that may be important in cardioprotection is its effect on vascular tone generation. Evidence of this would be estrogen improvement of coronary blood flow in both postmenopausal women (2) and monkeys with coronary atherosclerosis (3). The specific mechanism by which estrogen affects vascular tone still remains unclear.

Rabbit aorta data have shown that estrogen promotes and enhances endothelial-dependent relaxation and contraction (4,5). Additionally, an increase in endothelial nitric oxide (6) and prostacycline (7) release has been demonstrated in rabbit coronary arteries and rat aortas respectively. There is also the possibility that estrogen may influence vascular relaxation via decreasing endothelium-derived vasoconstrictor prostaglandins, thomboxanes (5), superoxide radicals and endothelines (8). Conversely, endothelium-independent relaxations are possible, as has been shown in the rabbit coronary artery (9). This relaxation and that in guinea pig cardiac myocytes (10) are thought to be due to calcium channel blocking properties of the hormone.

In this study, we investigated the vasoactivity of acute estrogen treatment in isolated bovine coronary arteries to add evidence in support of estrogen as an important cardiovascular agent. We also attempted to define the specific mechanisms responsible for this vasoactivity by employing the use of various pharmacological agents.

Materials and methods

Tone measurements

Bovine hearts were obtained from a local slaughterhouse right after slaughter. The left anterior descending coronary arteries were cut out and during transport they were kept in an ice-cold phosphate buffer (mM/liter) solution of: Glucose 11.09 mM/liter, NaCl 125, KCl 2.7, Tris 23.8 and CaCl2 2.0.

The vessels were cleaned carefully from surrounding tissue and cut, with a new scalpel, into rings of 2 to 3 mm in diameter and 2 mm in length. Care was taken to avoid damage to the endothelium. Some of the rings were denuded of endothelium by gentle rubbing of the lumen with the wooden handle of a cotton swab for about 30 sec. The arterial rings were then mounted on wire hooks attached to force displacement transducers (T 43-05, Colbourn Instruments) for measuring changes in isometric force using methods already described (11). Thereafter, vessels were incubated in 10 ml baths (Metro Scientific) with Krebs-buffer

(pH 7.4) solution containing (mM/liter): 118 NaCl, 4.7 KCl, 1.5 CaCL2, 25 NaHCO3, 1.1 MgSO4, 1.2 KH2PO4 and 5.6 glucose. The baths were individually thermostated (37°C), and gassed with 95% O_2/ balanced air. Rings were adjusted to 5g passive tension, which was found to be the optimal passive force for maximal contraction. Changes in force were recorded on a Colbourn computer-based recording system.

After a two-hour equilibration at the optimal passive tension, the vessels were depolarized with Krebs-bicarbonate solution containing KCl (123 M/liter) in place of NaCl. This treatment produces maximal contraction and enhances the reproducibility of subsequent contractions. Additionally, it allows the evaluation of the viability of vessel rings. The arteries were then re-equilibrated with Krebs-bicarbonate for 15 min. before conducting experiments. The removal of endothelium in some artery rings was confirmed by examining the effect of 0.01-1 (M acetylcholine on arteries precontracted with 0.1-3(μ/l 5-hydroxytryptamine (5HT). Endothelium-denuded arteries contracted at the largest dose of acetylcholine and relaxations were not observed.

Exposure to estradiol

Vessels were first precontracted to a submaximal average tone of 1.5 (0.3g using the thromboxane mimetic U_{46619} (10-100(M/liter). The dose of U_{46619} was adjusted to produce similar tone in all rings. Tone was allowed to achieve a steady state level; then, cumulative doses of estradiol (0.01-30μM/liter) were added to the organ baths, allowing a 5 min. period between doses or maximum response. After the highest dose was added, the experiment was completed.

In order to elucidate specific mechanisms of relaxation, the same experiments were again performed in the presence of a variety of pharmacological inhibitors for 15 min. These agents were chosen to block specific mechanisms that might contribute to specific estrogen effect.The concentrations and names of the agents were as follows: 10mM/L indomethacin (Indo, cyclooxygenase inhibitor), 10mM/L methylene blue (MB inhibits soluble guanylate cyclase), 100mM/L nitro-L-arginine (NLA inhibits nitric oxide synthesis), 1 mmol/L isobutylmethylxanthine (IBMX, cAMP phosphodiesterase inhibitor) or 30mM/L mifepristone (RU_{38486}, steroid receptor antagonist).

Dilutions of 5HT were made in distilled deionized water and 10μl aliquots were added to the 10 ml baths. Indomethacin was dissolved in absolute ethanol and 10μl from this solution was added to the bath. 17β-estradiol was dissolved in ethyl alcohol to give a stock solution of 10^{-2} M. Vehicle control with 10μl ethyl alcohol was performed in five vessels without any significant effect. The stock solutions of U_{44619} were made in ethyl alcohol and serial dilutions of stock were made in distilled deionized water. NLA, RU_{38486}, IBMX and MB were dissolved in distilled deionized water. NLA and IBMX were added in 100μl aliquots, whereas RU_{38486} and MB were added in 30- and 10μl aliquots respectively.

Materials

Indomethacin, methylene blue, nitro-L-arginine, 5HT (5-hydroxytryptamine), isobutylmethylxanthine and 17β-estradiol were purchased from Sigma Chemical Company (St. Louis). RU_{486} was generously provided by Roussel-UCLAF (Romainville, France). U^{44619} was obtained from Cayman Chemical, Ann Arbor, MI. Other chemicals were analyzed reagent grade from Baker Chemical Co. (Phillipsburg, NJ).

Statistical analysis

All relaxations were calculated as percent of U_{44619} induced tone. Nonpaired Student's t-test was utilized to compare responses between two groups. For multiple comparisons, analysis of variance (ANOVA) was performed followed by post-hoc Duncan's test.

The accepted level of significance was $p < 0.05$. The number of experimental determinations (n) in all cases is equal to the number of animals from which a vessel ring was used for treatment or control group.

Results

The addition of 0.01-30μmol/liter 17β-estradiol to intact bovine coronary vessels precontracted with 0.3μmol/liter U_{46619} causes rapid and sustained concentration-dependent relaxation up to 68% (n=15). The relaxation is expressed as a percentage of the U_{46619} induced tone before addition of the first estrogen dose.

In addition, comparison of the magnitude of relaxation in response to 17β-estradiol at each concentration level between endothelium-intact and endothelium-denuded arteries did not show significant difference (Figure 1). Thus, in our study, 17β-estradiol relaxation is not dependent on mediators released from the endothelium.

Bovine coronary vessels pretreated with inhibitors [(a) 10μmol/liter indomethacin, (b) 100μmol/liter nitro -L-arginine or (c) 10μmol/liter methyl blue] when exposed to 0.01 -30μol/liter 17β-estradiol were relaxed in a similar manner to vessels in the control group without inhibitors pretreatment (n= 15). The lack of effects of these probes is shown in Figure 2 (a,b,c). These results suggest that relaxation of bovine coronary vessels to 17(-estradiol is not mediated by relaxant prostaglandins, l-arginine products, or cGMP.

Pretreatment of bovine coronary vessels with 100mM/liter IBMX significantly enhanced the 17b-estradiol-induced relaxation from 20% to 40% ($n=15$ $p < 0.01$) as shown in Figure 3. This is consistent with mediation by cAMP.

Pretreatment of bovine coronary arteries with 30μM/liter RU_{38486} markedly reduced the relaxation to 17β-estradiol by more than 50% ($n=15$, $p < 0.05$) (Figure 4) suggesting a role for estrogen receptors in the mechanism of this relaxation.

Discussion

Animal studies in vitro and in vivo, as well as clinical studies, have suggested a variety of vascular effects and mechanisms of action of estrogen. This study shows that

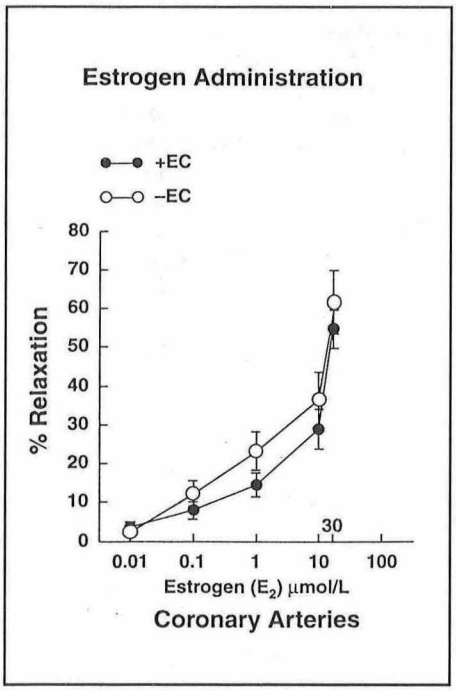


Figure 1. Summary data showing relaxation response to 17β-estradiol in endothelium-intact (+EC) and endothelium denuded (EC-) bovine coronary arteries and the lack of effect of removal of endothelium on the relaxation. The vessels were precontracted with thromboxane mimetic U_{46619}. The relaxation is given as a percentage of the U_{46619} induced tone and values represent the means ± S.E.M. in 15 determinations from 15 different animals.

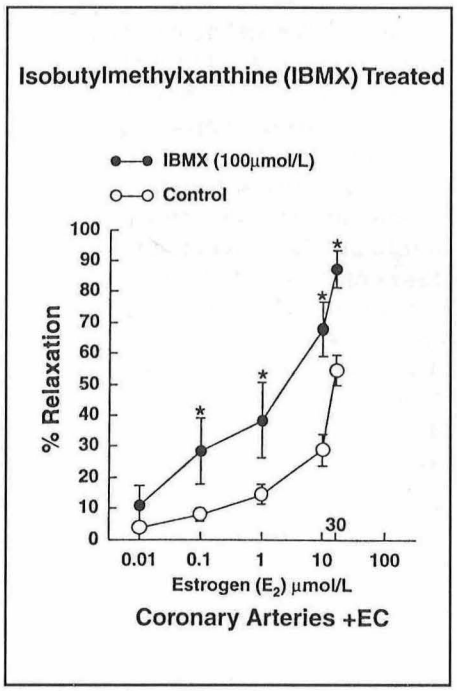


Figure 3. Summary data showing the enhancement of the relaxation from 20%-40% of endothelium-intact coronary bovine arteries in response to 17β-estradiol after pretreatment with 100µmol/L isobutylmethylxanthine (IBMX) suggest a role for cAMP (n=15, p<0.01).

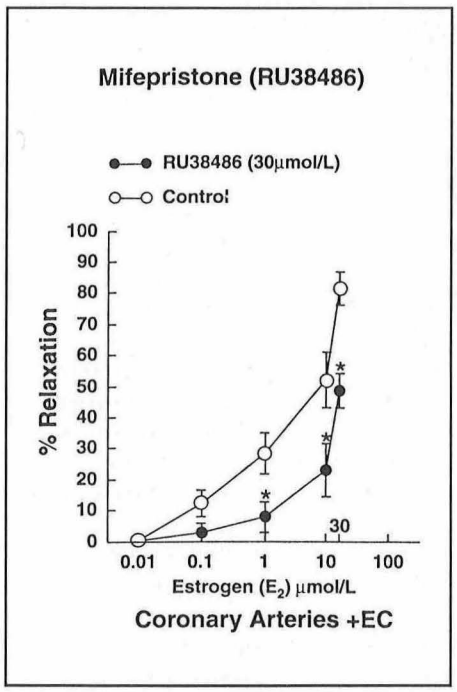
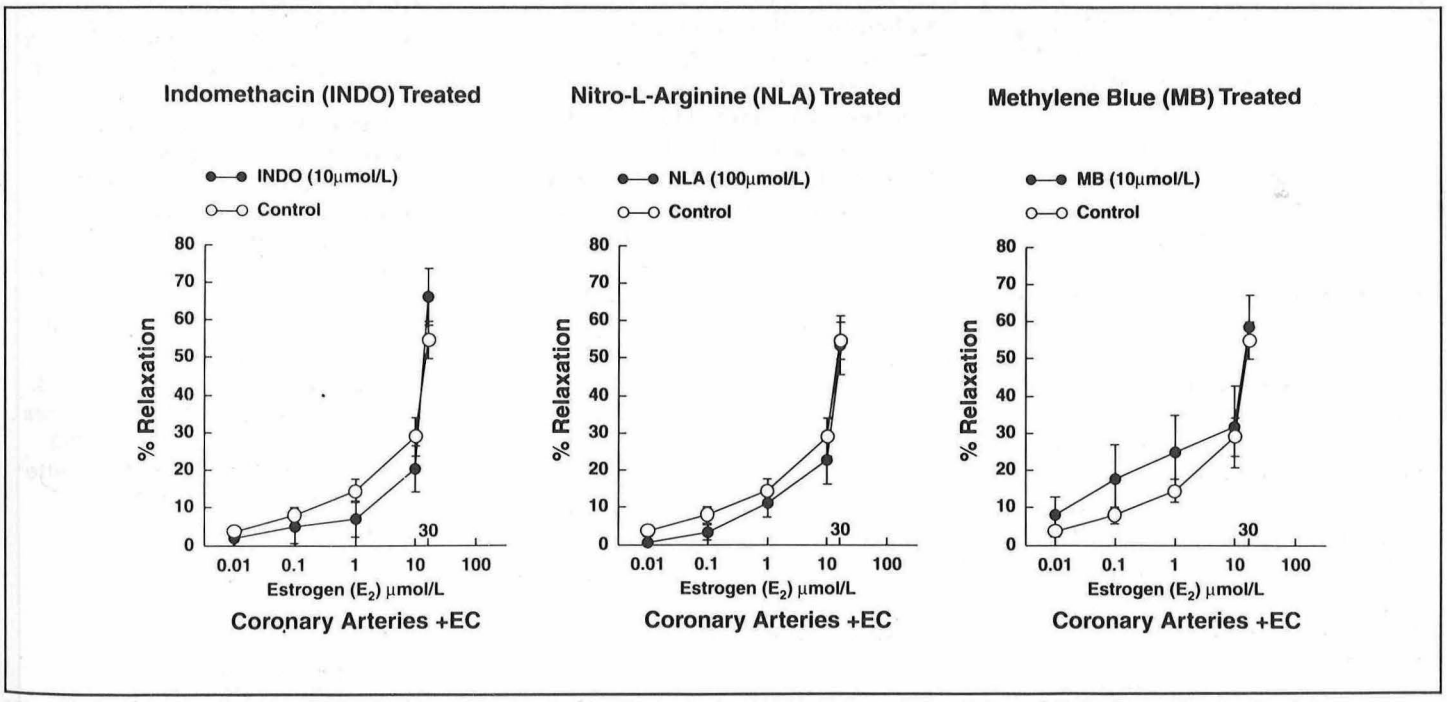


Figure 4. Summary data showing the inhibitory effect of pretreatment with 30µmol/L mifepristone (Ru_{38486}) on relaxation produced by 17β-estradiol in endothelium-intact bovine coronary arteries. Relaxation was reduced by more than 50% suggesting that estrogen receptors could be included (n=15, p<0.05).



Figures 2a,b,c. Summary data showing the lack of effect of pretreatment with (a) 10µmol/L Indomethacin (Indo); (b) 100µmol/L Nitro-L-Arginine (NLA); and (c) 10µmol/L Methyl blue (MB) on the relaxation produced by estrogen in endothelium-intact bovine coronary arteries (n=11-16).

17β-estradiol is a vasoactive hormone causing a rapid and sustained dose-dependent relaxation in precontracted bovine coronary arteries with and without endothelium.

Our results did not indicate a difference in relaxation between endothelium-intact and endothelium-denuded coronary bovine arteries. Furthermore, nitro-l-arginine and indomethacin, inhibitors of endothelium-derived relaxing factor and prostaglandin production, did not affect the relaxation induced by 17β-estradiol in endothelium-intact coronary arteries. The lack of effect of these probes in our study suggests that relaxation does not appear to be mediated by the endothelium via nitric oxide production or arachidonic acid metabolites.

Similar results have been reported in isolated rabbit coronary arteries (9) and on human atherosclerosis-free epicardial arteries in vitro (12). Another study showed that 17β-estradiol relaxation in rabbit coronary artery rings was endothelium and nitric oxide dependent under certain hormonal conditions such as acute estrogen withdrawal (13). A report suggesting the existence of both endothelium-dependent and endothelium-independent mechanisms (but at higher concentrations), in isolated rabbit aortic rings has also been published (14).

Potentiation of 17β-estradiol-induced relaxation by IBMX, as shown in our study, suggests a role for cAMP. IBMX, a phosphodiesterase inhibitor, may decrease cAMP metabolism, increase cAMP level and, through cAMP-dependent protein kinase-mediated events (15), enhance 17β-estradiol relaxation of bovine coronary arteries. Other authors have only suggested that cAMP might be involved in the cellular response to estrogen (16).

Methylene blue in our study did not affect relaxation to estradiol on the bovine coronary vessels, indicating no role for cGMP in this relaxation. However, in human coronary vessels, contents of both cAMP and cGMP were increased after exposure to estrogen (17). This could be explained by cross activation of cGMP-dependent protein kinase by cAMP. The elevation of cAMP within its physiological concentration range causes cGMP protein kinase-dependent activation in pig coronary smooth muscle cells. Thus, the smooth muscle relaxant effects of either cAMP or cGMP could be mediated by cGMP protein kinase-dependent activation (18).

It is unclear if the relaxation to 17β-estradiol involves changes in calcium influx as has been suggested in experiments on rabbit coronary rings (9) and rat aortic rings (19). A primary effect of estrogen on coronary arteries may involve Ca^{2+} and voltage-activated K^{+} channels (16). A portion of relaxation may reflect direct inhibition of potential sensitive and receptor-operated calcium channels as also has been suggested for uterine arteries (20).

Cyclic AMP has been reported to increase the efflux of Ca^{2+} from smooth muscle strips within minutes after its addition (21). Since cAMP increases intracellular pH and increased pH stimulates the Ca^{2+} pump, it is possible that such alkalization could be responsible for the cAMP-dependent increase of the Ca^{2+} extrusion from the cell (22).

In our experiments, RU^{38486} significantly inhibited the relaxation of bovine coronary vessels in response to 17β-estradiol, which is consistent with a need for estrogen receptor activation to elicit relaxation. Estrogen receptors have already been found in rat coronary artery smooth muscle cells (23), but the rapid vasorelaxation induced by high concentrations of estrogen excludes a genomic mechanism in the nucleus and indicates the possibility of non-genomic cell surface membrane binding sites (estrogen receptor) as have already been found at the outer surface of endometrial cells (24).

The physiological nanomolar concentrations of 17β-estradiol were unable to produce significant relaxation of coronary arteries in vitro. The relaxation is achieved with concentrations approaching the micromolar range. The experimental physiological solution does not contain steroid binding proteins. To more closely simulate physiological conditions, a higher concentration of free hormones is required. Thus, we used doses up to 30μM/liter.

Conclusion

Based on our study, 17β-estradiol causes a dose-dependent relaxation in bovine coronary arteries that does not appear to utilize endothelium, prostaglandins, cGMP or arginine products, but may involve cAMP and possibly estrogen receptors. We have described a novel mechanism of estrogen relaxation that previously has only been suggested (19). The same mechanism of vasorelaxation was presented for progesterone, another steroid hormone, in placental human vessels in vitro (25).

Since the 17β-estradiol-induced relaxation is endothelium-independent, treatment by 17β-estradiol may be used as a protection against myocardial ischemia in patients with atherosclerotic vessels.

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