Estrogen-Induced Relaxation in Bovine Coronary Arteries in vitro: Evidence for a New Mechanism

Jelica D. J. Kalenic  
*West Virginia University*

Rolando J. Ramirez  
*Magee-Womens Research Institute*

Stanley Einzig  
*West Virginia University*

William A. Neal  
*West Virginia University*

Hatim A. Omar  
*University of Kentucky*, hatim.omar@uky.edu

Follow this and additional works at: [https://uknowledge.uky.edu/pediatrics_facpub](https://uknowledge.uky.edu/pediatrics_facpub)

Part of the Pediatrics Commons

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Repository Citation
Kalenic, Jelica D. J.; Ramirez, Rolando J.; Einzig, Stanley; Neal, William A.; and Omar, Hatim A., "Estrogen-Induced Relaxation in Bovine Coronary Arteries in vitro: Evidence for a New Mechanism" (2000).  
*Pediatrics Faculty Publications*. 150.  
[https://uknowledge.uky.edu/pediatrics_facpub/150](https://uknowledge.uky.edu/pediatrics_facpub/150)

This Article is brought to you for free and open access by the Pediatrics at UKnowledge. It has been accepted for inclusion in Pediatrics Faculty Publications by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
Estrogen-Induced Relaxation in Bovine Coronary Arteries in vitro: Evidence for a New Mechanism

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

The copyright holder has granted permission for posting the article here.

This article is available at UKnowledge: https://uknowledge.uky.edu/pediatrics_facpub/150
Estrogen-induced relaxation in bovine coronary arteries in vitro: Evidence for a new mechanism

JELICA D. J. KALENIC, M.D.
Dept. of Pediatrics, West Virginia University School of Medicine, Morgantown

ROLANDO J. RAMIREZ, Ph.D.
Magee-Womens Research Institute, Pittsburgh, Pa.

STANLEY EINZIG, M.D.
Depts. of Pediatrics and Physiology, West Virginia University School of Medicine, Morgantown

WILLIAM A. NEAL, M.D.
Depts. of Pediatrics, West Virginia University School of Medicine, Morgantown

HATIM A. OMAR, M.D.
Dept. of Pediatrics, University of Kentucky, Chandler Medical Center, Lexington

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 U46619, were given doses (0.01-30 μM) of 17β-estradiol in the presence and absence of endothelium and these inhibitors: 10 μM indomethacin (cyclooxygenase inhibitor), 10 μM methylene blue (inhibits soluble guanylate cyclase), 100 μM nitro-L-arginine (inhibits nitric oxide synthesis), 100 μM isobutylmethylxanthine (phosphodiesterase inhibitor) and 30 μM mifepristone (RU38486, steroid receptor antagonist). Our results indicated that, estrogen, in the highest concentration used (30 μM), 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, thromboxanes (5), superoxide radicals and endothelins (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% O2/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 0.01-10 M. acetylcholine on arteries precontracted with 0.1-3 (u/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 U46619 (10-100M/liter). The
dose of U46619 was adjusted to
produce similar tone in all rings.
Tone was allowed to achieve a steady state level; then, cumulative
doses of estradiol (0.01-30[mu]M/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 (RU38486,
steroid receptor antagonist).

Dilutions of 5HT were made in
distilled deionized water and 10 [mu]l
aliquots were added to the 10 ml
baths. Indomethacin was dissolved in
absolute ethanol and 10 [mu]l from
this solution was added to the bath. 17]-estradiol was dissolved in ethyl
alcohol to give a stock solution of
10-6 M. Vehicle control with 10 [mu]l
ethyl alcohol was performed in five
vessels without any significant
effect. The stock solutions of U46619
were made in ethyl alcohol and
serial dilutions of stock were made in
distilled deionized water. NLA, RU38486, IBMX and MB were dissolved in
distilled deionized water. NLA and
IBMX were added in 100 [mu]l aliquots,
whereas RU38486 and MB were added in 30- and 10 [mu]l aliquots respectively.

Materials

Indomethacin, methylene blue, nitro-L-arginine, SHT
(5-hydroxytryptamine),
isobutylmethylxanthine and 17β-
estriol were purchased from Sigma
Chemical Company (St. Louis). RU38486
was generously provided by Roussel-
UCLAf (Romainville, France). U46619
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 U46619 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 0.01-30[mu]mol/liter
17β-estradiol to intact bovine
coronary vessels precontracted with
0.3[mu]mol/liter U46619 causes rapid and sustained concentration-dependent
relaxation up to 68% (n = 15). The
relaxation is expressed as a
percentage of the U46619 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)
10[mu]mol/liter indomethacin, (b)
100[mu]mol/liter nitro-L-arginine or (c)
10[mu]mol/liter methyl blue] when
exposed to 0.01-30[mu]mol/liter 17β-
estriol 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
to 17(-estradiol is not
mediated by relaxant prostaglandins,
L-arginine products, or cGMP.

Pretreatment of bovine coronary
to 17β-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 30[mu]M/liter RU38486
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
Estrogen Administration

[Graph showing relaxation response to 17β-estradiol in endothelium-intact (+EC) and endothelium-denuded (EC-) bovine coronary arteries.]

Isobutylmethylxanthine (IBMX) Treated

[Graph 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) suggests a role for cAMP (n=15, p<0.01).]

Mifepristone (RU38486)

[Graph showing the inhibitory effect of pretreatment with 30μmol/L mifepristone (RU38486) 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).]

Indomethacin (INDO) Treated

[Graph showing the lack of effect of pretreatment with 10μmol/L Indomethacin (Indo); 100μmol/L Nitro-L-Arginine (NLA); and 10μmol/L Methylene 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 a 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 Ca2+ 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 Ca2+ from smooth muscle strips within minutes after its addition (21). Since cAMP increases intracellular pH and increased pH stimulates the Ca2+ pump, it is possible that such alkalinization could be responsible for the cAMP-dependent increase of the Ca2+ extrusion from the cell (22).

In our experiments, R1881 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.

**References**


(Please contact the first author for the other references in this article.)