17β-Estradiol attenuates vascular contraction through inhibition of RhoA/Rho kinase pathway

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고혈압 치료제에 의한 혈관 수축 조절

Golan DE et al.,
Principles of Pharmacology, 2007
Targeting Rho and Rho-kinase in the treatment of cardiovascular disease

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Isoflavone attenuates vascular contraction through inhibition of RhoA/Rho-kinase signaling pathway

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Abstract

Isoflavones decrease blood pressure, improve lipid profiles, and restore vascular function. We hypothesized that isoflavone attenuates vascular contraction by inhibiting RhoA/Rho-kinase signaling pathway. Rat aortic rings were denuded of endothelium, mounted in organ baths, and contracted with U46619, a thromboxane A2 analogue or KCl 30 min after the pretreatment with geriastin, daidzein or vehicle. We determined the phosphorylation level of the myosin light chain (MLC), myosin phosphatase targeting subunit 1 (MYPT1) and protein kinase C (PKC) -potentiated inhibitory protein.
Gender, sex hormones, and vascular tone

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Endothelium - independent

GENOMIC (Long term)

Estrogen

NON-GENOMIC (Acute)

Inhibition of Growth & Proliferation

Inhibition of Contraction
Hypothesis

We hypothesized that 17β-estradiol attenuates vascular contraction by inhibiting RhoA/Rho kinase signaling pathway in rat aorta.
Materials and Methods

Tension measurements

- Organ bath (20ml)
- Animal: SD rat (10 week), Thoracic aorta (4mm in length)
Fig. 1

(a) Effect of U46619 on tension in the presence of different concentrations of NaF.

(b) Effect of NaF on tension in the presence of different concentrations of U46619.

(c) Effect of KCl on tension in the presence of different concentrations of PDBu.

(d) Effect of PDBu on tension in the presence of different concentrations of KCl.

Key:
- Vehicle
- 10 μM E2
- 30 μM E2
- 100 μM E2

** denotes statistical significance.
Regulation of Smooth Muscle Contraction

MLCK
Ca·CaM

Myosin II RLC$_{20}$

MLCP

Contraction
Stress fibers
Cell Migration

$\frac{\text{MLCK activity}}{\text{MLCP activity}} \approx [\text{RLC}_{20}]_P$

*Physiol Rev 2003;83(4)*
Fig. 2

Panel a: Graph showing the effect of different concentrations of 17β-Estradiol on MLC20 phosphorylation in the presence of U46619. The x-axis represents time (min) ranging from 0 to 14, and the y-axis represents Tension (mN) ranging from 0 to 60. The curves represent Vehicle, 30 µM E₂, and 100 µM E₂, with significant differences indicated by **.

Panel b: Bar graph showing the % MLC20 phosphorylation in response to different concentrations of 17β-Estradiol (µM) in the presence of U46619 (30 nM). The x-axis represents 17β-Estradiol concentration (µM) ranging from 0 to 100, and the y-axis represents % MLC20 phosphorylation ranging from 0 to 35. Significant differences are indicated by ** and ##.

Panel c: Graph showing the effect of different concentrations of 17β-Estradiol on MLC20 phosphorylation in the presence of NaF. The x-axis represents time (min) ranging from 0 to 40, and the y-axis represents Tension (mN) ranging from 0 to 60. The curves represent Vehicle, 30 µM E₂, and 100 µM E₂, with significant differences indicated by **.

Panel d: Bar graph showing the % MLC20 phosphorylation in response to different concentrations of 17β-Estradiol (µM) in the presence of NaF (8.0 mM). The x-axis represents 17β-Estradiol concentration (µM) ranging from 0 to 100, and the y-axis represents % MLC20 phosphorylation ranging from 0 to 50. Significant differences are indicated by ** and ##.
Regulation of smooth muscle contraction

PKC-potentiated inhibitory protein of 17 kDa

(myosin phosphatase target subunit)

Somlyo and Somlyo, Physiol Rev 2003:83:1325
Fig. 3

(a) 17β-Estradiol (μM) vs. U46619 (30 nM) effect on GTP RhoA. 
(b) 17β-Estradiol (μM) vs. NaF (8.0 mM) effect on GTP RhoA.
Fig. 4

(a) 17β-Estradiol (μM)  
- - 30 100  

(b) U46619 (30 nM)  
- + + +  

p-MYPT1^Thr855 →  

p-MYPT1^Thr855 →  

t-MYPT1 →  

t-MYPT1 →  

**  
**  
##  

Fold increase over control
0.0 1.0 2.0 3.0 4.0  

Fold increase over control
0.0 1.0 2.0 3.0 4.0  

p-MYPT1^Thr855 →  

p-MYPT1^Thr855 →  

**  
**  
##  

17β-Estradiol (μM)  
- - 30 100  

NaF (8.0 mM)  
- + + +  

*  

**  

ab  

ab  

**  

ab  

**  

ab  

**  

ab  

ab
Fig. 5

(a) 17\(^{\beta}\)-Estradiol (\(\mu\)M)  
-  -  30  100  
U46619 (30 nM)  
-  +  +  +

(b) 17\(^{\beta}\)-Estradiol (\(\mu\)M)  
-  -  30  100  
NaF (8.0 mM)  
-  +  +  +
Regulation of smooth muscle myosin phosphatase

PKC

\[ \text{p-CPI17}_{\text{Thr38}} \]

Rho-kinase

ILK, ZIPK

MLCP inhibition

\[ \text{Thr697 Thr855} \]

Modified from Molecular & Cellular Biochemistry 259:197-209, 2004
Fig. 8

a

17β-Estradiol (100 μM)     Ro31-8220 (1.0 μM)            PDBu (0.1 μM)              
Fold decrease over PDBu
0.0
0.2
0.4
0.6
0.8
1.0
1.2
**
-
- +
- -
+
-
+
-
+ +-

17β-Estradiol (100 μM)     Ro31-8220 (1.0 μM)            PDBu (0.1 μM)              
Tension (mN)
0
10
20
30
40
50
60
**

17β-Estradiol (100 μM)
Ro31-8220 (1.0 μM)
PDBu (0.1 μM)
+
+
+
+

b

p-CPI17Thr^{38} →

p-CPI17/t-CPI17 Ratio

Fold decrease over PDBu

** **
-
- +
- -
+
-
+
-
+ +-

p-CPI17/t-CPI17 Ratio

Fold decrease over PDBu

** **
-
- +
- -
+
-
+
-
+ +-

17β-Estradiol (100 μM)     Ro31-8220 (1.0 μM)            PDBu (0.1 μM)              
+
-
-
+

17β-Estradiol (100 μM)     Ro31-8220 (1.0 μM)            PDBu (0.1 μM)              
+
-
-
+

17β-Estradiol (100 μM)     Ro31-8220 (1.0 μM)            PDBu (0.1 μM)              
+
-
-
+
Summary

• $17\beta$-Estradiol attenuated vascular tension induced by U46619, NaF or KCl, but not PDBu.

• $17\beta$-Estradiol decreased not only the activation of RhoA, but also $\text{MLC}_{20}$ phosphorylation induced by U46619 or NaF.

• $17\beta$-Estradiol also decreased the level of phosphorylation of MYPT1 and CPI17 induced by U46619 or NaF.

• $17\beta$-Estradiol did not affect vasocontraction and CPI17 phosphorylation induced by PDBu.
Conclusion

17β-Estradiol attenuates vascular contraction through inhibition of RhoA/Rho kinase pathway.
Thank you for your attention!
Gender, sex hormones, and vascular tone

Julia M. Orshal and Raouf A. Khalil
Research and Development, Department of Veterans Affairs Medical Center, West Roxbury; and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02132

Endothelium - dependent

Endothelial Cell

Smooth Muscle Cell
Multiple signaling pathways of estrogen in cardiovascular cells

Fulvestrant (Faslodex; ICI 182780)
Reverse effect of estrogen receptor antagonist ICI 182,780 on the 17β-estradiol induced vasorelaxation in rat aorta.
Selective Estrogen Receptor agonist

DPN
(4,40,400-(4-propyl-[1H]-pyrazole-1,3,5-triyl) tris-phenol)

PPT
(2,3-bis(4-hydroxyphenyl)-propionitrile)

ERα agonist

ERβ agonist
Inhibitory effects of 17β-estradiol, PPT and DPN on U46619-induced vasocontraction in rat aorta
Fig. 6

(a) U46619 (-log mol/L) vs. Tension (mN)
(b) NaF (mM) vs. Tension (mN)
(c) KCl (mM) vs. Tension (mN)
(d) PDBu (-log M) vs. Tension (mN)
Fig. 7

(a) NaF (mM) vs. Tension (mN)
(b) Vehicle vs. NaF (mM)
(c) U46619 (-log M) vs. Tension (mN)
(d) PDBu (-log M) vs. Tension (mN)

KCl (mM) vs. Tension (mN)

** denotes statistical significance at the p < 0.01 level.
Divergent effects of estrogen and nicotine on Rho-kinase expression in human coronary vascular smooth muscle cells
**Figures a and b**

**a**

- p-MYPT1\(^{Thr855}\) → t-MYPT1
- NaF (6.0 mM)
- H1152 (μM)
- Y27632 (μM)

**Fold increase over control**

- 0.3
- 1
- 3

**b**

- p-CPI17\(^{Thr38}\) → t-CPI17
- NaF (6.0 mM)
- H1152 (μM)
- Ro318220 (μM)

**Fold increase over control**

- 0.3
- 1
- 3

**Legend**

**a** and **b** show the fold increase over control for p-MYPT1\(^{Thr855}\) and p-CPI17\(^{Thr38}\) respectively, under different concentrations of NaF, H1152, Y27632, Ro318220.
Regulation of smooth muscle contraction

1. GPCR activates PLC, which hydrolyzes PIP_2 to DAG and IP_3.
2. IP_3 influxes Ca^{2+} into the cell.
3. Ca^{2+} activates CaM and PKC.
4. CaM activates CPI-17, which activates CPI-17-P.
5. CPI-17-P activates Rho-kinase.
7. RhoA-GTP activates RhoGEF, which activates RhoA-GTP.
8. RhoA-GTP activates RhoA-GDP, which inhibits ATP.
10. RhoA-GTP activates MLCP-P, which dephosphorylates MLC and deactivates MLCP.
11. MLCP-P dephosphorylates MLC, which deactivates MLCK.
12. MLCK deactivates MLC-P, which deactivates Contraction.
13. Contraction

Postmenopausal Hypertension Mechanisms and Therapy

Matthias Barton, Matthias R. Meyer

Table. Effects of Postmenopausal HT on Blood Pressure and Atherosclerotic Vascular Disease

<table>
<thead>
<tr>
<th>Potentially Beneficial</th>
<th>Potentially Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT using 17β-estradiol</td>
<td>HT using animal estrogens (CEEs)</td>
</tr>
<tr>
<td>Transdermal administration of HT</td>
<td>Oral administration of HT</td>
</tr>
<tr>
<td>Begin of HT early after menopause</td>
<td>Begin of HT late after menopause</td>
</tr>
<tr>
<td>Low dosage of HT</td>
<td>High dosage of HT</td>
</tr>
<tr>
<td>Cyclic administration of HT</td>
<td>Progestins with adverse effects (MPA)</td>
</tr>
</tbody>
</table>
G_{12}\text{-}G_{13}\text{-}LARG\text{-}mediated signaling in vascular smooth muscle is required for salt-induced hypertension

Angela Wirth\(^1\), Zoltán Benyó\(^{1,2,7}\), Martina Lukasova\(^{1,7}\), Barbara Leutgeb\(^{1,6}\), Nina Wettschureck\(^1\), Stefan Gorbey\(^3\), Petra Örly\(^1\), Béla Horváth\(^1\), Christiane Maser-Gluth\(^1\), Erich Greiner\(^{4,6}\), Björn Lemmer\(^3\), Günther Schütz\(^4\), J Silvio Gutkind\(^5\) & Stefan Offermanns\(^1\)

**Diagram:**
- Diagram shows various labeled areas including a cell type specific promoter, Cre, loxP, and target gene
- Text highlights the relationship between 
  - DOCA-salt and blood pressure changes in different genotypes (WT, Sm-q-11-KO, Sm-12-13-KO)
  - Tamoxifen effect on blood pressure
- Graphs illustrate blood pressure changes over time with DOCA-salt treatment

**Figure e:**
- Diagram of signaling pathways involving GPCR, PLC-\(\beta\), G_{q-11}, G_{12-13}, and LARG
- Pathways include:
  - IP\(_3\):
  - \([\text{Ca}^{2+}]\):
  - MLC:
  - MLCP:
  - Rho:
  - ROCK:
- Basal blood pressure and Salt-induced hypertension

**Notes:**
- Diagrams and graphs support the text by illustrating the mechanisms of action and the effects of genetic modifications on blood pressure regulation.
G_{12}-G_{13}-LARG-mediated signaling in vascular smooth muscle is required for salt-induced hypertension

Angela Wirth^1, Zoltán Benyó^1,2,7, Martina Lukasova^1,7, Barbara Leutgeb^1,6, Nina Wettschureck^1, Stefan Gorbey^3, Petra Örsy^1, Béla Horváth^1, Christiane Maser-Gluth^1, Erich Greiner^4,6, Björn Lemmer^3, Günther Schütz^4, J Silvio Gutkind^5 & Stefan Offermanns^1
Estrogen should reduce development of hypertension through peripheral actions such as up-regulation of endothelium-derived vasodilator factors with simultaneous down-regulation of vasoconstrictor factors, such as endothelin-1 (Barber et al., 1996; Barber and Miller, 1998; Best et al., 1998; Dubey et al., 2001), inhibition of the renin-angiotensin system by reducing transcription of angiotensin-converting enzyme in endothelial cells (Brosnihan et al., 1994; Gallagher et al., 1999), and down-regulation of angiotensin 1 receptors (Nickenig et