The Mechanism of Endothelium-Independent Relaxation Induced by the Wine Polyphenol Resveratrol in Human Internal Mammary Artery

Aleksandra Novakovic et al.
University of Belgrade, Serbia and Montenegro
Journal of Pharmacological Sciences, 2006
Presented by Wiraporn Paebua SIPS/M

Resveratrol (Trihydroxystilbene)

- Phenolic substance presenting in Polygonum cuspidatum roots and Grapes
- Firstly discovered by Siemann and Creasy in 1992

Ion channels of vascular smooth muscle cells

- L-type, T-type, ROC, Na⁺/Ca²⁺
- K⁺, KATP, Kᵣ, Kᵢ
- Vascular smooth muscle cell
- NSCC: nonspecific cation channel

Relaxation of smooth muscle cells

Endothelium-dependent manners

- Ach
- NO
- L-Arg
- L-citrulline
- GTP
- cGMP
- Vascular smooth muscle cell
- Na⁺
- Ca²⁺

Cis- and trans-isomer form of resveratrol

- Trans-resveratrol
- Cis-resveratrol

Ca²⁺
K⁺
L-Arg
GTP
cGMP
Kᵣ
Vascular smooth muscle cell
Endothelium
L-citrulline
NO
GTP
Kᵣ
Endothelium-independent manners

Relaxation of smooth muscle cells

K+ channels openers

Nitric oxide donors

Hyperpolarization

Vascular smooth muscle

Potassium channel blockers

Glibenclamide: Selective blocker of K_{ATP} channels

Charybdotoxin: BK_{ca} channel blocker, also block K_{v} 1.2, 1.3 channels at high doses

TEA: Selective blocker of BK_{ca} channels

4-AP: K_{v} channels blocker

Margatoxin: Selective inhibitor of K_{v} 1.1, 1.2, 1.3, 1.8 channels

Introduction

• Recent study showed an inverse correlation between red wine consumption and incidence of cardiovascular diseases
• Resveratrol might be partly responsible for the cardiovascular benefits associated with wine consumption
• The mechanism of cardiovascular benefits probably includes vasorelaxation, antioxidant, and anti-platelet effects of resveratrol

(Chan and Pace-Asciak, 1996)

Human internal mammary artery (HIMA)

• The internal mammary artery, previously called as the internal thoracic artery, originating from the subclavian artery at the root of the neck on each side

Coronary Artery Bypass Graft (CABG)

• HIMA is the vessel of choice for coronary artery bypass graft CABG
• Endothelium of bypass graft can be damaged due to blunt surgical trauma
• The importance of vasodilating substances were investigated in HIMA with denuded endothelium

Potassium channel openers, pinacidil, levcromakalim, rilmakalim
AIMS OF THIS STUDY

- To examine the vasodilatating effects of resveratrol on the HIMA
- To define the contribution of different K+ channel subtypes in endothelium-independent resveratrol action on this blood vessels

Materials and Methods

No calcium antagonists and long-acting nitrates administration for 48 hrs
Clamping the blood flow and excised
Placing in cold Krebs-Ringer bicarbonate solution

Materials and methods

Equilibration (30 mins)

Equilibrium response
Washed 3 times
Variety of K+ blockers

Treatment of data and statistics

- Percentage of the maximum possible relaxation
- EC50
- Means ± S.E.M

By using the Computer program Graph Pad Prism
The molar concentration of an agonist, which produces 50% of the maximum possible response for that agonist

\[ EC_{50} \] was calculated from

\[ pD_2 = -\log EC_{50} \]

**Drugs**

- Trans-resveratrol
- Phenylephrine
- Acetylcholine
- Glibenclamide
- Charybdotoxin
- 4-aminopyridine (4-AP)
- Tetraethylammonium chloride (TEA)
- Margatoxin

**Results**

**Effects of resveratrol on precontracted HIMA**

- Max. = 83 ± 1%
- \( pD_2 = 4.38 \pm 0.11 \)

**Effects of potassium channel antagonists on the resveratrol-induced relaxation of HIMA**

**Glibenclamide (10 μM)**

- In the absence of glibenclamide
  - Maximal response = 83 ± 2%
  - \( pD_2 = 4.39 \pm 0.24 \)
- In the presence of glibenclamide
  - Maximal response = 83 ± 5%
  - \( pD_2 = 4.36 \pm 0.12 \), P>0.05

**Charybdotoxin (10 nM)**

- In absence of charybdotoxin
  - Maximal response = 83 ± 2%
  - \( pD_2 = 4.36 \pm 0.31 \)
- In presence of charybdotoxin
  - Maximal response = 81 ± 2%
  - \( pD_2 = 4.32 \pm 0.23 \), P>0.05

**TEA (3 mM)**

- In absence of TEA
  - Maximal response = 83 ± 1%
  - \( pD_2 = 4.31 \pm 0.22 \)
- In presence of TEA
  - Maximal response = 82 ± 2%
  - \( pD_2 = 4.38 \pm 0.13 \), P>0.05

It did not significantly modify the relaxation of HIMA induced by resveratrol.
Discussion

• Previous study
  Resveratrol inhibited KCl, noradrenaline-, and phenylephrine-induced contraction of rat aorta and mesenteric artery

  The mechanism of endothelium-independent vasorelaxation has not been defined

Glibenclamide (10 μM)

Glibenclamide is the most selective blockers of K_{ATP} channels

It may block some other types of K⁺ channels when used in a high concentration (>30 μM)

In this study:

K_{ATP} channels are not involved in the pathway by which resveratrol produces a relaxation of the HIMA

Discussion

TEA (3 mM)

TEA was found to selectively block the BK_{Ca} channels in concentrations lower than 1 mM (Kd = 0.29 mM)

It can block other K⁺ channels in concentration up to 10 mM

In this study:

BK_{Ca} channels are not involved in resveratrol-induced relaxation of the HIMA
Discussion

Charybdoxin-sensitive channels are not involved in the mechanism of resveratrol-induced relaxation of the HIMA

**Charybdoxin (10 nM)**

Charybdoxin is not a specific blocker of BK<sub>Ca</sub> channels

It also inhibits intermediate conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels and Kv channels (1.2 and 1.3 subtypes)

**In this study:**

Charybdoxin-sensitive channels are not involved in the mechanism of resveratrol-induced relaxation of the HIMA

Discussion

**4-AP (3 mM)**

4-AP achieved some selectivity for Kv channels used in low millimolar concentration

It has been shown to be without effect on BK<sub>Ca</sub> channels

**In this study:**

Activation of Kv channels are involved in the mechanism of resveratrol-induced relaxation of the HIMA

Discussion

**Margatoxin (10 nM)**

Margatoxin is a highly selective inhibitor of the Kv channels, especially 1.1, 1.2, 1.3, and 1.6 subtypes

It displays no affinity for the mammalian BK<sub>Ca</sub> channels

**In this study:**

Kv 1.1, 1.2, 1.3 and 1.6 subtypes might be included in the mechanism of resveratrol-induced relaxation of HIMA

Discussion

The fact that charybdoxin also blocks Kv1.2 and 1.3 channels and did not reproduce the effect of margatoxin suggests that

**Kv1.1 and/ or Kv1.6 channels may be the relevant target**

Conclusion

Resveratrol induces relaxation of the denuded HIMA rings

**4-AP and margatoxin-sensitive K<sup>+</sup> channels in vascular smooth muscle mediated the relaxation of HIMA produced by resveratrol**

THANK YOU FOR YOUR ATTENTION

(c) 2008 www.cuketsa.cz
L-NAME

• (NG-monomethyl L-arginine, a NOSi)
  non-selective inhibitor of nitric oxide synthase used experimentally to induce hypertension