Kidney Res Clin Pract 33 (2014) 181–186

Kidney Research and Clinical Practice

journal homepage: http://www.krcp-ksn.com
Contents lists available at ScienceDirect

Original Article

Mechanisms of phytoestrogen biochanin A-induced vasorelaxation in renovascular hypertensive rats

Seok Choi [1], Won Suk Jung [2], Nam Soo Cho [2], Kwon Ho Ryu [1], Jae Yeoul Jun [1], Byung Chul Shin [3], Jong Hoon Chung [3], Cheol Ho Yeum [1,*]

[1] Department of Physiology, College of Medicine, Chosun University, Gwangju, Korea
[2] Department of Emergency Medicine, College of Medicine, Chosun University, Gwangju, Korea
[3] Department of Internal Medicine, College of Medicine, Chosun University, Gwangju, Korea

Article history:
Received 17 June 2014
Received in revised form
20 August 2014
Accepted 26 August 2014
Available online 12 November 2014

Keywords:
Biochanin A
Endothelium
K^+ channels
Renovascular hypertension

Abstract

Background: The plant-derived estrogen biochanin A is known to cause vasodilation, but its mechanism of action in hypertension remains unclear. This study was undertaken to investigate the effects and mechanisms of biochanin A on the thoracic aorta in two-kidney, one clip (2K1C) renovascular hypertensive rats.

Methods: Hypertension was induced by clipping the left renal artery, and control age-matched rats were sham treated. Thoracic aortae were mounted in tissue baths to measure isometric tension.

Results: Biochanin A caused concentration-dependent relaxation in aortic rings from 2K1C hypertensive and sham-treated rats, which was greater in 2K1C rats than in sham rats. Biochanin A-induced relaxation was significantly attenuated by removing the endothelium in aortic rings from 2K1C rats, but not in sham rats. N^{ω}-Nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor, or indomethacin, a cyclooxygenase inhibitor, did not affect the biochanin A-induced relaxation in aortic rings from 2K1C and sham rats. By contrast, treatment with glibenclamide, a selective inhibitor of adenosine triphosphate-sensitive K^+ channels, or tetraethylammonium, an inhibitor of Ca^{2+}-activated K^+ channels, significantly reduced biochanin A-induced relaxation in aortic rings from both groups. However, 4-aminopyridine, a selective inhibitor of voltage-dependent K^+ channels, inhibited the relaxation induced by biochanin A in 2K1C rats, whereas no significant differences were observed in sham rats.

Conclusion: These results suggest that the enhanced relaxation caused by biochanin A in aortic rings from hypertensive rats is endothelium dependent. Vascular smooth muscle K^+ channels may be involved in biochanin A-induced relaxation in aortae from hypertensive and normotensive rats. In addition, an endothelium-derived activation of voltage-dependent K^+ channels contributes, at least in part, to the relaxant effect of biochanin A in renovascular hypertension.

Introduction

Estrogen replacement therapy markedly reduces the risk of cardiovascular disease in postmenopausal women [1,2]. However, the use of hormone replacement therapy as a cardioprotective

* Corresponding author. Department of Physiology, College of Medicine, Chosun University, 309, Pilmundaero, Dong-Gu, Gwangju 501-759, Korea.
E-mail address: chyum@chosun.ac.kr (CH Yeum).

2211-9132/$ - see front matter © 2014. The Korean Society of Nephrology. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
http://dx.doi.org/10.1016/j.krcp.2014.08.003

strategy is greatly limited owing to carcinogenic effects in women and feminizing effects in men. Phytoestrogens are naturally occurring plant-derived nonsteroidal estrogens, which also bind to the estrogen receptor, acting as estrogen agonists or antagonists [3,4].

It has been shown that dietary soy-derived estrogens are vasoactive and have beneficial cardiovascular effects [5]. Both dietary estrogens [6] and estradiol replacement therapy [7] enhance the vasorelaxant response to acetylcholine in female monkeys, whereas systemic arterial compliance was improved by a red clover diet in menopausal women [8]. Biochanin A that naturally occurs in soybeans and clover is estrogen-like [9]. It has been shown to relax the rabbit basilar artery [10] and to induce significant sex-independent relaxation of rabbit coronary arteries [11]. Furthermore, Wang et al [1] subsequently confirmed that biochanin A induces endothelium-independent relaxation in rat aortic rings, of which mechanism involves blockage of Ca^{2+} entry through the cell membrane and activation of K^+ channels. In addition, biochanin A-induced vasorelaxation was increased in spontaneously hypertensive rats than in normotensive rats, which was mediated by the release of endothelium-derived substances that may open voltage-dependent and Ca^{2+}-activated K^+ channels in the vascular smooth muscle [12]. Under these backgrounds, we have previously shown that an activation of Ca^{2+}-activated K^+ channels in vasorelaxation is altered in two-kidney, one clip (2K1C) renal hypertension [13]. Although an endothelium-derived activation of smooth muscle cell K^+ channels, which contributes to biochanin A-induced vasorelaxation, was observed in spontaneously hypertensive rats, the effect of biochanin A on vascular function in 2K1C renovascular hypertension remains unclear.

The present study was designed to examine the mechanisms of the relaxing effects induced by biochanin A in the thoracic aorta isolated from 2K1C renovascular hypertensive rats.

Methods

Induction of 2K1C renovascular hypertension

Renovascular hypertension was induced in rats following the 2K1C Goldblatt model [14]. Briefly, male Sprague–Dawley rats (Samtako Inc., Osan, Korea), weighing 160–180 g, were anesthetized with an intraperitoneal injection of sodium thiopental (40 mg/kg). Under antiseptic conditions, an incision was made on the left flank to access the left renal artery, which was separated from the renal vein and cleaned of connective tissue. A U-shaped solid silver clip with an internal diameter of 0.2 mm was applied to the exposed renal artery, resulting in the partial occlusion of renal perfusion. The contralateral kidney remained untouched, and the wound was closed. A group of age-matched rats were sham treated and served as the control group. The control animals underwent an operation similar to the 2K1C rats except that no clip was used. All animals were fed normal chow and were given tap water. They were used for the experiments at 10 weeks after the clipping, because endothelial dysfunction is associated with the duration of hypertension [15]. Hypertensive rats were selected based on their systolic blood pressure measured in a conscious state by the tail cuff method.

Tissue preparation

The thoracic aorta between the aortic arch and diaphragm was carefully removed and placed in cold, standard physiological salt solution with the following composition: NaCl 118.3mM, KCl 4.7mM, $NaHCO_3$ 25mM, $MgCl_2$ 1.2mM, KH_2PO_4 1.2mM, $CaCl_2$ 2.5mM, and glucose 11.1mM. Vessels were cleaned of adherent fat and connective tissue, and then cut into 2–3 mm long cylindrical rings under a dissecting microscope. The rings were suspended between two triangle-shaped stainless steel holders in the vessel lumen in organ baths containing 15 mL physiological salt solution maintained at $37 \pm 0.05^{\circ}C$, aerated with a mixture of 95% O_2 and 5% CO_2, and maintained at a pH of 7.4 ± 0.01. One of the holders was fixed at the bottom of the chamber, and the other was connected to a Grass force displacement transducer (FTO3, Quincey, MA, USA) to measure isometric tension development. Prior to initiating specific experimental protocols, the aortic rings were stretched to the point of their optimal length–tension relationship 2 g, which was determined in similar preliminary experiments using repeated exposure to 60mM KCl (obtained by equimolar replacement of NaCl by KCl in the physiological solution), and equilibration for a period of at least 90 minutes.

Protocols

At the beginning of the experiment, aortic rings were stimulated with 60mM KCl to test their functional integrity. In all experiments, aortic rings from 2K1C and sham-operated rats were precontracted to 50% effective concentration (EC_{50}) with phenylephrine (3×10^{-7}M in sham and 4×10^{-8}M in 2K1C), which were obtained in preliminary experiments. When the contractile response achieved a steady state, relaxation–response curves to the cumulative addition of biochanin A (from 10^{-7}M to 10^{-4}M), acetylcholine (from 10^{-9}M to 10^{-5}M), or sodium nitroprusside (SNP; from 10^{-10}M to $10^{-6.5}$M) were determined. In an alternate set of experiments, the aorta was pretreated for 10 minutes with biochanin A (3×10^{-5}M) prior to the addition of phenylephrine in the case of acetylcholine- and SNP-induced relaxation. To verify the role of functional endothelium in the vascular relaxant effects of biochanin A, the endothelium was removed from some thoracic aortae by gently rubbing the intimal surface with a moistened cotton swab. The successful removal of the endothelial cells from aortic rings was confirmed via the inability of acetylcholine to induce relaxation.

In another experiments, the nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10^{-4}M) or the cyclo-oxygenase inhibitor indomethacin (10^{-5}M), and K^+ channel blockers glibenclamide (3×10^{-6}M), tetraethylammonium (TEA, 10^{-3}M), or 4-aminopyridine (10^{-3}M), were added to the bath 10 minutes prior to the addition of phenylephrine.

Drugs

Acetylcholine, 4-aminopyridine, biochanin A, glibenclamide, indomethacin, L-NAME, SNP, and TEA were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade. Biochanin A, glibenclamide, and indomethacin were dissolved in dimethylsulfoxide, and the others were prepared in distilled water. The final concentrations of dimethylsulfoxide were $< 0.05\%$, which did not alter the contraction or relaxation responses.

Statistical analysis

The values presented in the figures are the means and standard errors of the means. Relaxant responses are presented