Kidney Res Clin Pract 33 (2014) 19–25

Kidney Research and Clinical Practice

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

Original Article

Effects of oxidative stress on endothelial modulation of contractions in aorta from renal hypertensive rats

Seok Choi [1], Hye Rang Shin [2], Sang Hoon Kim [2], Mi Jung Lee [1], Jae Yeoul Jun [1], Hyun Lee Kim [3], Jong Hoon Chung [3], Cheol Ho Yeum [1,*]

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

Article history:
Received 26 September 2013
Received in revised form
4 December 2013
Accepted 4 December 2013
Available online 14 February 2014

Keywords:
Endothelium
Renal hypertension
Superoxide
Oxidative stress

Abstract

Background: Endothelial dysfunction is linked to exaggerated production of superoxide anions. This study was conducted to examine the effects of oxidative stress on endothelial modulation of contractions in chronic two-kidney, one-clip (2K1C) renal hypertensive rats.

Methods: The 2K1C hypertension was induced by clipping the left renal artery; age-matched rats receiving sham treatment served as controls. Thoracic aortae were isolated and mounted in tissue baths for measurement of isometric tension.

Results: Norepinephrine-induced contraction was augmented by the removal of the endothelium, which was more pronounced in sham rats than in 2K1C rats. Nω-nitro-L-arginine methyl ester, an inhibitor of nitric oxide production, had a similar augmenting effect. Vitamin C inhibited the contraction in aortic rings with intact endothelium from 2K1C rats but not from sham rats. The contraction was also suppressed by treatment with diphenyleneiodonium or apocynin, inhibitors of nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidase, in the aortae with intact endothelium from 2K1C rats but not in those from sham rats. Superoxide anions generated by xanthine oxidase/hypoxanthine enhanced the contraction in the aortae with intact endothelium from sham rats, but had no effect in 2K1C rats. Enhanced contractile responses to norepinephrine by xanthine oxidase/hypoxanthine in sham rats were reversed by vitamin C.

Conclusion: These results suggest that the effect on endothelial modulation of endothelium-derived nitric oxide is impaired in 2K1C hypertension. The impairment is, at least in part, related to increased production of superoxide anions by NADH/NADPH oxidase.

Introduction

Initially regarded as an inert lining between blood and blood vessels, the vascular endothelium has a critical role in the maintenance of vascular tone and blood pressure by generating various vasoactive substances [1]. In physiological states, the endothelium releases both relaxing and contracting factors;

* 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.2013.12.001

a balance between these two factors contributes to the local regulation of vascular tone [2]. It has been known that hypertension is characterized by endothelial dysfunction in large conduit and small resistance arteries [3,4]. Endothelium-dependent vasodilation is indeed impaired in a number of experimental models, including two-kidney, one-clip (2K1C) hypertension [5–7]. Reduced production of endothelial relaxing factors or increased production of endothelial contractile substances may be responsible for the depressed endothelium-dependent relaxation in hypertension [8].

In addition to the direct relaxing or contracting effects of the endothelium, it can also modulate the effects of vasoconstrictor substances. The important role of the vascular endothelium in the response of isolated vascular segments to several vasoconstrictors, including norepinephrine, is widely recognized [6,9]. Constrictors apparently interact with the endothelium and induce the release of relaxing factors, which then have an inhibitory effect on vascular smooth muscle tone [6,10]. In our previous study, we demonstrated that the endothelium plays an inhibitory role against contractile responses to norepinephrine by releasing nitric oxide (NO), and the endothelial inhibition is attenuated in 2K1C hypertension [11]. Nevertheless, the mechanisms underlying the impaired endothelial modulation in hypertension remain to be established.

An endothelial dysfunction is known to be linked to the exaggerated production of superoxide anions. It has been proposed that increased oxidative inactivation of NO caused by an excess of superoxide anions is responsible for reducing the bioavailability of NO, which is in part related to endothelial dysfunction in hypertension [12,13]. Miyagawa et al [14] reported that vascular oxidative stress could contribute to an altered circulation by impairment of endothelial modulation of vascular contractions in spontaneously hypertensive rats. In addition, an increase in oxidative stress systemically plays a major role in the maintenance of high arterial blood pressure and sympathetic drive in renal hypertension [15]. We also observed that hydrogen peroxide may contribute in part to the altered vascular relaxation in 2K1C hypertensive rats [16].

The current study was undertaken to determine whether oxidative stress is involved in the impaired endothelial modulation of vascular contractions in 2K1C renal hypertension.

Methods

Induction of 2K1C renal hypertension

Male Sprague-Dawley rats (160–180 g) were anesthetized with sodium thiopental (40 mg/kg, IP). The left posterior side of the animal was shaved and sterilized with 70% ethanol. An incision of approximately 2 cm in length was made through the skin and muscles just below the ribs. The left kidney was then exposed and retracted in order to expose the renal artery, and a silver clip (internal diameter 0.2 mm) was applied to the exposed renal artery. The clip was then turned such that the slit opening was facing the abdomen. The contralateral kidney was left intact. The muscles and skin were then sutured, and the animal was left to recover from anesthesia. Control rats received a sham treatment, which involved exactly the same procedure except that no clip was placed. Postoperatively, all animals were fed normal chow and given tap water. They were operated at 10 weeks after clipping, because endothelial dysfunction is associated with the duration of hypertension [17]. Hypertensive rats were selected based on systolic blood pressure measured in a conscious state using the tail-cuff method and a piezoelectric pneumotaxic pulse transducer.

Tissue preparation

The thoracic aorta between the aortic arch and diaphragm was removed rapidly and placed in a physiological salt solution of 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 adhering tissue and cut into rings (2 mm wide) under a dissection microscope. Care was taken not to stretch the artery or damage the luminal surface. In some preparations, the endothelium was removed by gentle rubbing of the intimal surface with a moist cotton swab. Successful removal of endothelial cells from aortic rings was confirmed by the inability of acetylcholine to induce relaxation.

The aortic rings were suspended using two triangular-shaped stainless-steel holders in the vessel lumen in organ chambers containing 15 mL of physiological salt solution maintained at 37°C and aerated with 95% O_2 and 5% CO_2. To measure isometric tension development, one of the holders was fixed at the bottom of the chambers and the other was connected to a force displacement transducer (FTO3; Grass Technologies, Warwick, RI, USA). Prior to initiating specific experimental protocols, aortic rings were stretched to the point of their optimal length–tension relationship, which was determined to be 2 g by similar preliminary experiments involving repeated exposure to 60mM KCl solution (obtained by equimolar replacement of NaCl by KCl in physiological solution), and allowed to equilibrate for 90 minutes. After equilibration, rings were maximally contracted by the 60mM KCl solution to test their contractile capacity.

Protocols

During the first set of experiments, contractile responses to norepinephrine (10^{-10}–10^{-5}M) were determined in rings with or without the endothelium. To obtain α-adrenoceptor-mediated responses to norepinephrine, aortic rings were pretreated with a β-adrenoceptor antagonist timolol (3×10^{-7}M). To confirm the role of NO in the endothelium-dependent modulation of vascular contraction, concentration–response curves of norepinephrine were obtained in the presence of Nω-nitro-L-arginine methyl ester (L-NAME; 10^{-4}M), which inhibits the endogenous production of NO from L-arginine.

In the second set of experiments, to verify the role of oxidative stress and the involvement of nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidase in endothelial dysfunction in 2K1C rats, effects of vitamin C (10^{-4}M) or inhibitors of NADH/NADPH oxidase, diphenyleneiodonium (DPI; 10^{-5}M), or apocynin (3×10^{-5}M) on the contractile responses to norepinephrine [14] were determined. In order to assess the potential role of other enzymatic sources of superoxide anions, we recorded the concentration–response to norepinephrine of aortic rings pretreated with allopurinol (3×10^{-4}M), a xanthine oxidase inhibitor.

In the third set of experiments, using the hypoxanthine–xanthine oxidase system, which generates superoxide anions and hydrogen peroxides [18], we examined the effects of