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Reduced effects of endothelium-derived hyperpolarizing factor in ocular ciliary arteries from spontaneous hypertensive rats

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ABSTRACT

Vascular peripheral resistance is increased during hypertension, and endothelium-derived hyperpolarizing factor (EDHF) is an important for regulating vasodilation in small arteries. Therefore we characterized the role of EDHF in regulating vascular resistance of ocular ciliary arteries from spontaneous hypertensive rats (SHR) and age-matched Wistar Kyoto rats (WKY). Isometric tension recordings were used in isolated ocular ciliary artery segments from SHR and WKY. Ocular ciliary arteries pre-contracted with 100 μM norepinephrine exhibited a concentration-dependent relaxation to acetylcholine, and the effect on SHR arteries was smaller than that on WKY arteries ($P < 0.05$). The EDHF-mediated component of this relaxation, determined in the presence of 100 μM L-NAME plus 10 μM indomethacin, was also smaller in SHR than in WKY arteries ($P < 0.05$). Apamin (1 μM), a blocker of small-conductance calcium-activated K^+ (K_{Ca}) channels, had no effect on EDHF-mediated relaxation in either preparation. However, charybdotoxin (0.1 μM), which blocks intermediate- and large-conductance K_{Ca} channels, and iberiotoxin (0.1 μM), which blocks large-conductance K_{Ca} channels, almost completely suppressed EDHF-mediated relaxation in both preparations. The tension of ciliary arteries from both SHR and WKY was increased above baseline by 100 μM L-NAME plus 10 μM indomethacin. In these preparations, apamin had no effect on the tension in arteries from either SHR or WKY. However, both charybdotoxin and iberiotoxin further increased tension above that induced by L-NAME and indomethacin. The increase was smaller for SHR than WKY ($P < 0.05$). In summary, the ability of EDHF to relax ocular ciliary artery vascular tone in SHR is smaller than in WKY. The large-conductance calcium-activated K^+ channel is utilized in EDHF-signaling pathway.

1. Introduction

Increased vascular peripheral resistance contributes to elevated blood pressure in hypertensive patients (Franz, 1991) and experimental animals (Boegehold et al., 1991). The vascular endothelium is a major regulator of vascular tone, and changes in the endothelium could increase peripheral resistance (Boulanger, 1999). Vascular endothelial cells can release nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF), all of which act as vasodilators. All three relaxing factors are decreased in hypertensive patients and in animal models of hypertension, and the term "endothelial dysfunction" has been used to describe this association (Boulanger, 1999).

EDHF is an important vasodilator in resistance vessels (Brandes et al., 2000), and in spontaneous hypertensive rats (SHR), EDHF-mediated vasodilatation of femoral resistance arteries is attenuated (Mori et al., 2006). EDHF causes hyperpolarization of vascular smooth muscle cells in different preparations. Unlike NO and prostacyclin, the biochemical nature of EDHF remains controversial when compared among different species and vascular beds (Dalsgaard et al., 2009; Edwards et al., 1999; Gauthier et al., 2005). The EDHF hyperpolarization signaling pathway may be mediated through calcium-activated K^+ (K_{Ca}) channels (Doughty et al., 1999; Campbell et al., 1996). Activation of small- and intermediate-conductance K_{Ca} (SK_{Ca} and IK_{Ca}) channels present in endothelial cells (Eichler et al., 2003) can hyperpolarize vascular smooth muscle cells through gap junctions (Edwards et al., 1999) or by potassium ions (Edwards et al., 1998).

Arterial hypertension decreases end organ blood flow, resulting in pathogenic changes. In the retinas of hypertensive patients, perifoveal

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capillary blood flow, measured by scanning laser ophthalmoscopy, is significantly reduced compared with the normotensive subjects (Wolf et al., 1994). Likewise, systolic and diastolic flow velocities of the central retinal and posterior ciliary arteries, measured by duplex scanner, are decreased in patients with hypertension (Steigerwalt et al., 1998). To clarify the mechanism underlying reduced ocular blood flow in hypertension, we measured isometric tension in isolated ocular ciliary arteries from SHR and age-matched Wistar Kyoto rats (WKY) and determined the effects of EDHF on arterial relaxation. We also measured the effect of EDHF on the maintenance of resting tone.

2. Methods and materials

2.1. Animals

Male SHR and WKY were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. This study was approved by the Animal Experiment Committee of Akita University.

2.2. Measurement of cardiovascular variables and isolation of the ocular ciliary arteries

The heart rate (HR), systolic and diastolic blood pressures (SBP and DBP) of 20-week-old rats were measured by a tail-cuff fitted with an electro-sphygmomanometer (BP-98A; Softron, Tokyo, Japan). Afterward, the rats were killed with an overdose of ethyl ether (Abbott, North Chicago, IL, USA), and the heart and body weights were measured. The eyes were immediately enucleated, ensuring that a maximum length of optic nerve was removed, and then placed in aerated (95% O_2 and 5% CO_2) Krebs solution of the following composition (mM): NaCl 94.8, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.7. Using a dissecting microscope, the ciliary artery and surrounding connective tissue were carefully isolated from the optic nerve.

The detailed procedure for mounting the ciliary arterial segments in the Myograph System® (JP Trading, Aarhus, Denmark) has been described in our previous reports (Dong et al., 2006, 2007, 2008). Briefly, vascular segments (150–300 μm in diameter, 1–2 mm in length) were cut from the distal section of the ciliary artery, immediately mounted in the myograph chamber, and immersed in 10 ml Krebs solution. The temperature was raised to 37 °C and maintained for the duration of the study. The vessels were stretched to an internal diameter, I_1, that was 0.9 of the diameter generated by transmural pressure of 100 mmHg. This was the optimal diameter for active tension development (Halpern et al., 1978). After the vessels were equilibrated for 30 min, the experiments were performed. The Myograph System® directly determined vessel isometric tension and simultaneously transmitted the data to a computer that displayed the tension curves on a monitor. Detailed methods for isometric tension recordings by the Myograph System® have been described by Mulvany and Halpern (1976, 1977).

2.3. Protocols

After the equilibration period, contractions evoked by a high-K solution were maintained during a 20-min interval. The high-K solution was prepared by replacing NaCl with isotonic, equimolar KCl to give a final K^+ concentration of 100.7 mM. After 20 min, 1 μM carbachol, a cholinergic agonist that acts on receptors in the endothelium, was applied to induce relaxation of vascular smooth muscle (Keef and Bowen, 1989). This procedure established the susceptibility of each contracted ciliary artery to endothelium-dependent relaxation. Preparations in which high-K-induced contraction was less than 3 mN or carbachol-induced relaxation was less than 10% of 100 μM norepinephrine-induced contraction were excluded from this study. After establishing the responsiveness of the arterial segment, the high-K medium was washed out with Krebs solution.

In arteries, muscarinic agonists such as acetylcholine can stimulate the release of vasodilators, i.e. NO, prostacyclin, and EDHF, from the endothelium (Martinez-León et al., 2003). Thus, acetylcholine-induced endothelium-dependent relaxation was determined in segments that were pre-contracted with 100 μM norepinephrine. Generally, the norepinephrine-induced contraction was maintained for 20 min, then 10 nM–30 μM acetylcholine was applied every 10 min in a cumulative manner (Fig. 1A). The relaxation to acetylcholine was a transient response in ocular ciliary arteries of rats, and the statistical analysis for relaxation was performed with the peak relaxation for each dose of acetylcholine. Production of NO and prostacyclin induced by acetylcholine were blocked by inhibition of NO synthase and cyclooxygenase activity with 100 μM N^G-nitro-L-arginine methylester (L-NAME) and 10 μM indomethacin respectively. Under these conditions, the acetylcholine-induced relaxation of the arteries was attributable to the release of EDHF (Fig. 1B).

To determine if K_{Ca} channels were involved in EDHF-mediated relaxation, 1 μM apamin, a SK_{Ca} channel blocker (Pannirselvam et al., 2006), 0.1 μM charybdotoxin, an IK_{Ca} and a large-conductance K_{Ca} (BK_{Ca}) channel blocker (Quignard et al., 2000), or 0.1 μM iberiotoxin, a BK_{Ca} channel blocker (Quignard et al., 2000) was added in the presence of L-NAME and indomethacin. These additions were made prior to induction of vessel contraction by norepinephrine.

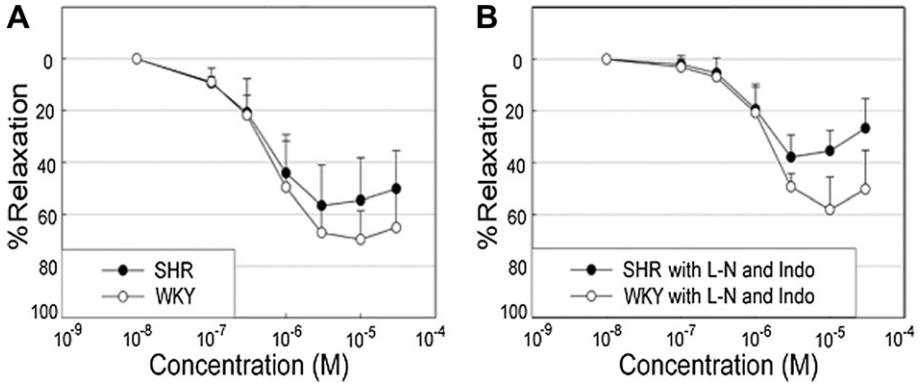

Fig. 1. Acetylcholine-induced relaxation in ocular ciliary arteries from spontaneous hypertension rats (SHR) and age-matched Wistar Kyoto rats (WKY). (A) Absence of L-NAME and indomethacin. (B) Presence of L-NAME and indomethacin. Significantly smaller relaxation occurred in SHR compared to WKY arteries ($P < 0.05$), with or without L-NAME and indomethacin.