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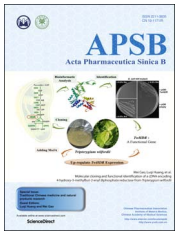

ORIGINAL ARTICLE

Arterial relaxation is coupled to inhibition of mitochondrial fission in arterial smooth muscle cells: comparison of vasorelaxant effects of verapamil and phentolamine

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Artery;
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Abstract Mitochondria are morphologically dynamic organelles which undergo fission and fusion processes. Our previous study found that arterial constriction was always accompanied by increased mitochondrial fission in smooth muscle cells, whereas inhibition of mitochondrial fission in smooth muscle cells was associated with arterial relaxation. Here, we used the typical vasorelaxants, verapamil and phentolamine, to further confirm the coupling between arterial constriction and mitochondrial fission in rat aorta. Results showed that phentolamine but not verapamil induced vasorelaxation in phenylephrine (PE)-induced rat thoracic aorta constriction. Verapamil, but not phentolamine, induced vasorelaxation in high K^+ (KPSS)-induced rat thoracic aorta constriction. Pre-treatment with phentolamine prevented PE- but not KPSS-induced aorta constriction and pre-treatment with verapamil prevented both PE- and KPSS-induced aorta constriction. Transmission electron microscopy (TEM) results showed that verapamil but not phentolamine inhibited KPSS-induced excessive mitochondrial fission in aortic smooth muscle cells, and verapamil prevented both PE- and KPSS-induced excessive mitochondrial fission in aortic smooth

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muscle cells. Verapamil inhibited KPSS-induced excessive mitochondrial fission in cultured vascular smooth muscle cells (A10). These results further demonstrate that arterial relaxation is coupled to inhibition of mitochondrial fission in arterial smooth muscle cells.

1. Introduction

Mitochondria are morphologically dynamic organelles which undergo fission and fusion dynamic processes. Mitochondrial dynamics are mainly regulated by mitochondrial fusion-related proteins including the outer mitochondrial membrane (OMM) proteins, mitofusin 1 (MFN1), mitofusin 2 (MFN2), the inner mitochondrial membrane (IMM) protein, optic atrophy factor 1 (OPA1), and fission-related proteins including dynamin-related protein 1 (DRP1), human fission factor-1 (Fis1), mitochondrial fission factor (MFF), MiD49 and MiD51[1].

Mitochondrial fission has been reported to be involved in apoptosis[2], autophagy[3], mitochondrial transport[4], cell differentiation[5], embryonic development[6] and metabolism[7]. Disorders of mitochondrial fission contribute to a variety of pathological processes. Mitochondrial fission has been implicated in diabetes[8], cardiomyocyte hypertrophy[9], myocardial ischemia/reperfusion injury[10], heart failure[11] and neurodegenerative disease[12,13]. Recently, some literature showed that regulation of mitochondrial fission might be a novel target to prevent cardiovascular diseases including hypertension, pulmonary arterial hypertension, atherosclerosis, and intimal hyperplasia[1,14–16].

Our previous study found that phenylephrine (PE)- and KPSS-induced vasoconstriction was accompanied by increased mitochondrial fission in smooth muscle cells, and mitochondrial fission inhibitors (mdivi-1 and dynasore) both inhibited vasoconstriction induced by PE or KPSS[17]. Furthermore, Y27632 (a ROCK inhibitor) and nitroglycerin relaxed KPSS-induced vasoconstriction and inhibited KPSS-induced mitochondrial fission[17]. These results indicated that there might be a coupling between arterial constriction and mitochondrial fission in smooth muscle cells. In order to confirm the hypothesis, we also used other typical vasorelaxants, verapamil and phentolamine, to examine the relationship between arterial constriction and mitochondrial fission in smooth muscle cells from rat thoracic aorta. Here, the effects of verapamil and phentolamine in vasoconstriction models induced by PE or KPSS further demonstrate that arterial relaxation is coupled to inhibition of mitochondrial fission in arterial smooth muscle cells.

2. Materials and methods

2.1. Agents and animals

Acetylcholine chloride (Ach) was purchased from Sigma-Aldrich Chemistry (Saint Louis, MO, USA). Mito-Tracker Green and Hoechst were purchased from Life Technology (Invitrogen, OR, USA). PE and verapamil were purchased from Harvest Pharmaceutical Co., Ltd. (Shanghai, China). Phentolamine was purchased from Santa Cruz Biotechnology, Inc. (Shanghai, China). Arterial smooth muscle cells (A10) were purchased from ATCC (VA, USA). Adult male Sprague-Dawley rats were purchased from Charles River (Charles River Laboratory Animal, Beijing, China). All animal procedures and experiments were approved by the Institutional Animal Care and Use Committee of Harbin Medical University. High K^+ salt solutions containing 60 and 50 mmol/L K^+ were used for treating arterial tissues and smooth muscle cells respectively. The KPSS (60 mmol/L K^+) solution was composed of (mmol/L): NaCl, 74.7; KCl, 60; $MgSO_4 \cdot 7H_2O$, 1.17; KH_2PO_4, 1.18; $NaHCO_3$, 14.9; $CaCl_2$, 1.6; D-glucose, 5.5; EDTA, 0.026. The KPSS (50 mmol/L K^+) solution was composed of (mmol/L): NaCl, 84.7; KCl, 50; $MgSO_4 \cdot 7H_2O$, 1.17; KH_2PO_4, 1.18; $NaHCO_3$, 14.9; $CaCl_2$, 1.6; D-glucose, 5.5; EDTA, 0.026.

2.2. Aorta tension measurement

The experiments were carried out according to our previous work[17,18]. Adult male Sprague-Dawley rats were sacrificed after anesthesia with sodium pentobarbitone. The thorax was cut to expose the aorta, and the descending thoracic aorta was rapidly dissected and transferred to physiological salt solution (PSS) at room temperature. After the perivascular tissue was carefully removed, aortic rings were cut approximately 4 mm in length and mounted between two stainless steel triangle hooks and then transferred to an organ bath with 10 mL fresh PSS solution oxygenated with 95% O_2 and 5% CO_2 (pH 7.4) at 37 °C. After equilibration, the tension was measured by using a multichannel acquisition and analysis system (Model BL-420E, Taimeng Technology Instrument, Chengdu, China).

2.3. Measurements of mitochondrial networks

The experiments were carried out according to our previous work[17]. Cultured arterial smooth muscle cells (A10) were loaded with Mito-Tracker Green (50 nmol/L) for 20 min and Hoechst (1 μg/mL) for 15 min at 37 °C. The cells were imaged by using the Zeiss LSM 700 confocal microscope (Carl Zeiss, Jena, Germany). All imaging was observed with a 40 × oil immersion objective lens. Mitochondrial fragmentation was analyzed according to literature[10]. Mitochondrial length was determined by use of Image-Pro Plus software.

2.4. Transmission electron microscopy (TEM)

The experiments were carried out according to our previous work[17]. Samples were rinsed in buffer, and then fixed in 2.5%

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