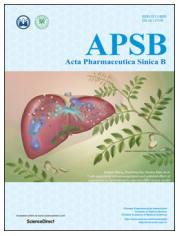
Acta Pharmaceutica Sinica B 2017;7(3):319–325

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

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

**Jing Jin[a,b,†], Xin Shen[a,b,†], Yu Tai[a,b], Shanliang Li[a,b], Mingyu Liu[a,b], Changlin Zhen[a,b], Xiuchen Xuan[a,b], Xiyue Zhang[a,b], Nan Hu[a,b], Xinzi Zhang[a,b], Deli Dong[a,b,*]**

[a]*Department of Pharmacology (the State-Province Key Laboratories of Biomedicine-Pharmaceutics of China, Key Laboratory of Cardiovascular Research, Ministry of Education), College of Pharmacy, Harbin Medical University, Harbin 150086, China*
[b]*Translational Medicine Research and Cooperation Center of Northern China, Heilongjiang Academy of Medical Sciences, Harbin Medical University, Harbin 150086, China*

Received 11 October 2016; received in revised form 9 November 2016; accepted 29 November 2016

**KEY WORDS**

Artery;
Mitochondrial fission;
Phentolamine;
Vasorelaxation;
Verapamil

**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

*Corresponding author. Tel.: +86 451 86671354; fax: +86 451 86667511.
E-mail address: dongdeli@ems.hrbmu.edu.cn (Deli Dong).
†These authors made equal contributions to this work.
Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2016.12.009
2211-3835 © 2017 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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.

© 2017 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

## 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%

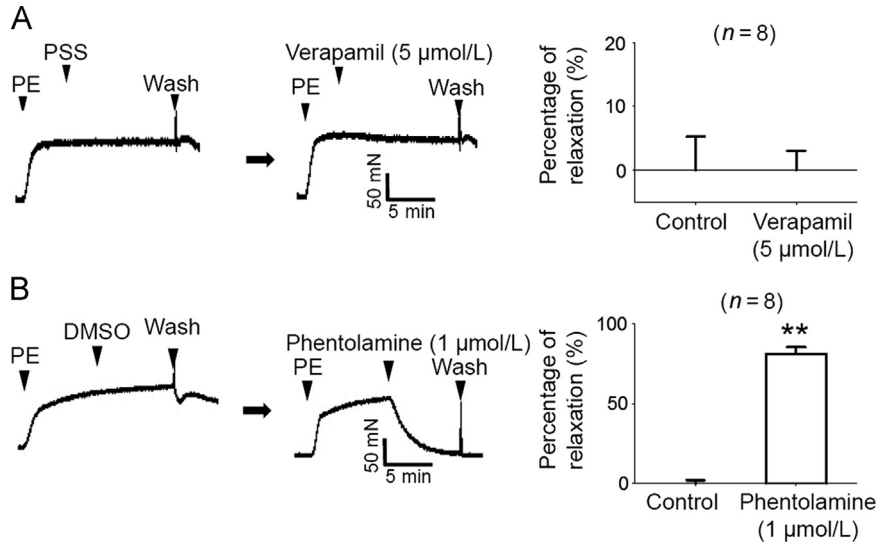



**Figure 1** Phentolamine but not verapamil induced vasorelaxation in PE-induced rat thoracic aorta constriction. (A) Verapamil (5 μmol/L) showed no effect on PE (1 μmol/L)-evoked rat thoracic aorta constriction. (B) Phentolamine (1 μmol/L) relaxed PE (1 μmol/L)-induced rat thoracic aorta constriction. $^{**}P<0.01$ *vs.* control (DMSO), $n=8$.

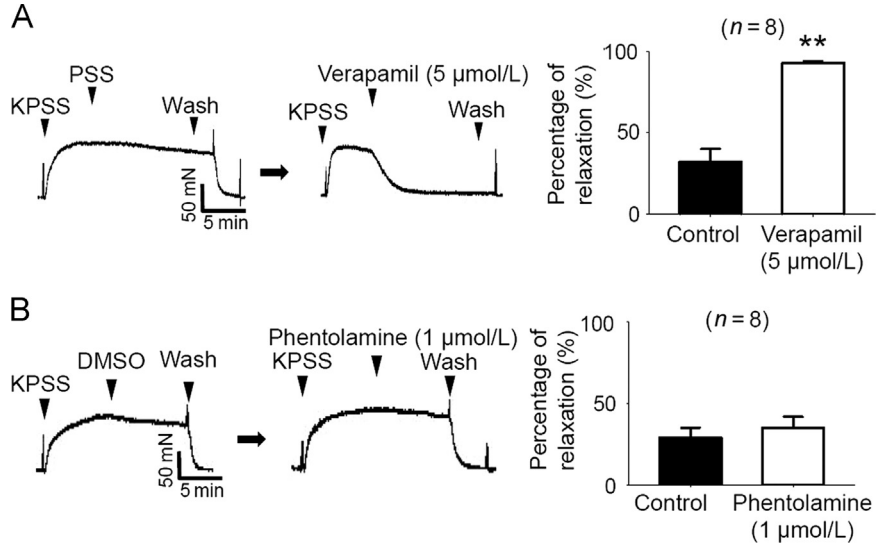



**Figure 2** Verapamil but not phentolamine induced vasorelaxation in KPSS-induced rat thoracic aorta constriction. (A) Verapamil (5 μmol/L) relaxed KPSS (60 mmol/L $K^+$)-evoked rat thoracic aorta constriction. $^{**}P < 0.01$ *vs.* control (PSS). (B) Phentolamine (1 μmol/L) showed no effect on KPSS (60 mmol/L $K^+$)-induced rat thoracic aorta constriction, $n=8$.

glutaraldehyde in PBS (pH 7.4) for 2–3 days. Specimens were then post-fixed in PBS-buffered 1% $OsO_4$ for 1–2 h, stained *en bloc* in uranyl acetate, dehydrated in ethanol, and embedded in epoxy resin by standard procedures. The ultrathin sections were electron stained and observed under an electron microscope (JEM-1220, JEOL Ltd., Tokyo, Japan).

### 2.5. *Data analysis*

Data are presented as mean ± SEM. Significance was determined by using Student's *t*-test or one-way ANOVA followed by Holm-Sidak. $P<0.05$ was considered significant.

## 3. Results and discussion

### 3.1. *Phentolamine induces vasorelaxation in PE-constricted rat thoracic aorta*

PE induces vasoconstriction through stimulating $\alpha_1$-adrenergic receptors in plasma membrane of smooth muscle cells and the subsequent activation of inositol 1,4,5-trisphosphate receptors (IP3Rs) on sarcoplasmic reticulum. As shown in Fig. 1, phentolamine but not verapamil induced vasorelaxation in PE-induced rat thoracic aorta constriction. Phentolamine is an $\alpha_1$-adrenergic receptor antagonist, and induced vasorelaxation *via* inhibition of PE-induced activation of $\alpha_1$-adrenergic receptors.

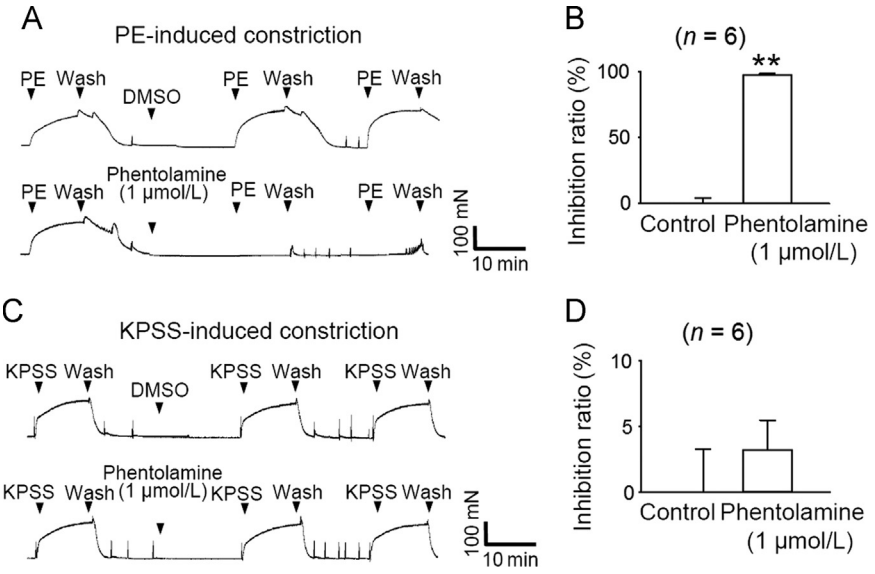



**Figure 3** Phentolamine pretreatment prevented PE- but not KPSS-induced aorta constriction. (A) and (B) Phentolamine (1 μmol/L) pretreatment inhibited PE (1 μmol/L)-induced aorta constriction. $^{**}P<0.01$ *vs.* control (DMSO). (C) and (D) Phentolamine (1 μmol/L) pretreatment exerted no effect on KPSS (60 mmol/L $K^+$)-evoked constriction of rat thoracic aorta, $n=6$.

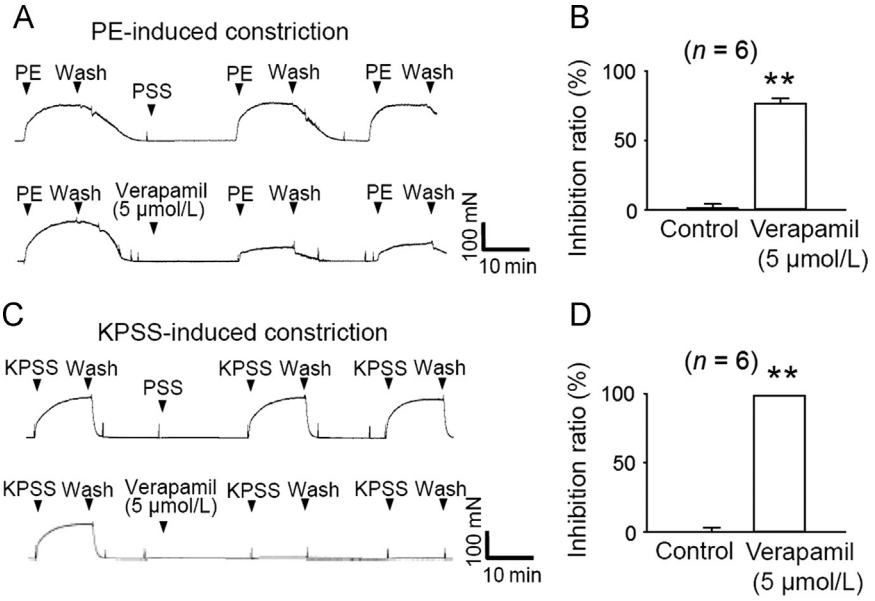



**Figure 4** Verapamil pretreatment prevented both PE- and KPSS-induced aorta constriction. (A) and (B) Verapamil (5 μmol/L) pretreatment inhibited PE (1 μmol/L)-induced constriction of rat thoracic aorta. $^{**}P<0.01$ *vs.* control. (C) and (D) Verapamil (5 μmol/L) pretreatment prevented KPSS (60 mmol/L $K^+$)-evoked constriction of rat thoracic aorta. $^{**}P<0.01$ *vs.* control, $n=6$.

### *3.2. Verapamil induces vasorelaxation in KPSS-constricted rat thoracic aorta*

KPSS induces vasoconstriction by depolarizing membrane potential of smooth muscle cells and the subsequent activation of L-type $Ca^{2+}$ channels. In the KPSS-induced rat thoracic aorta constriction model, verapamil but not phentolamine induced vasorelaxation (Fig. 2). Due to the different mechanisms of PE- and KPSS-induced vasoconstriction, $\alpha_1$-adrenergic receptor antagonist and $Ca^{2+}$ channel blocker showed distinct effects in these models.

### *3.3. The effect of pretreatment of phentolamine or verapamil on PE- or KPSS-induced aorta constrictions*

We further examined the preventive effects of phentolamine and verapamil on PE- and KPSS-induced aorta constriction. As shown in Fig. 3, pretreatment with phentolamine prevented PE- but not KPSS-induced aorta constriction. However, pretreatment with verapamil prevented both PE- and KPSS-induced aorta constrictions (Fig. 4). Accordingly, we suggest that the primary origin of intracellular $Ca^{2+}$ can be traced to extracellular $Ca^{2+}$ *via* $Ca^{2+}$ channels in vascular smooth muscle cells; hence the storage of intracellular $Ca^{2+}$ is suppressed by

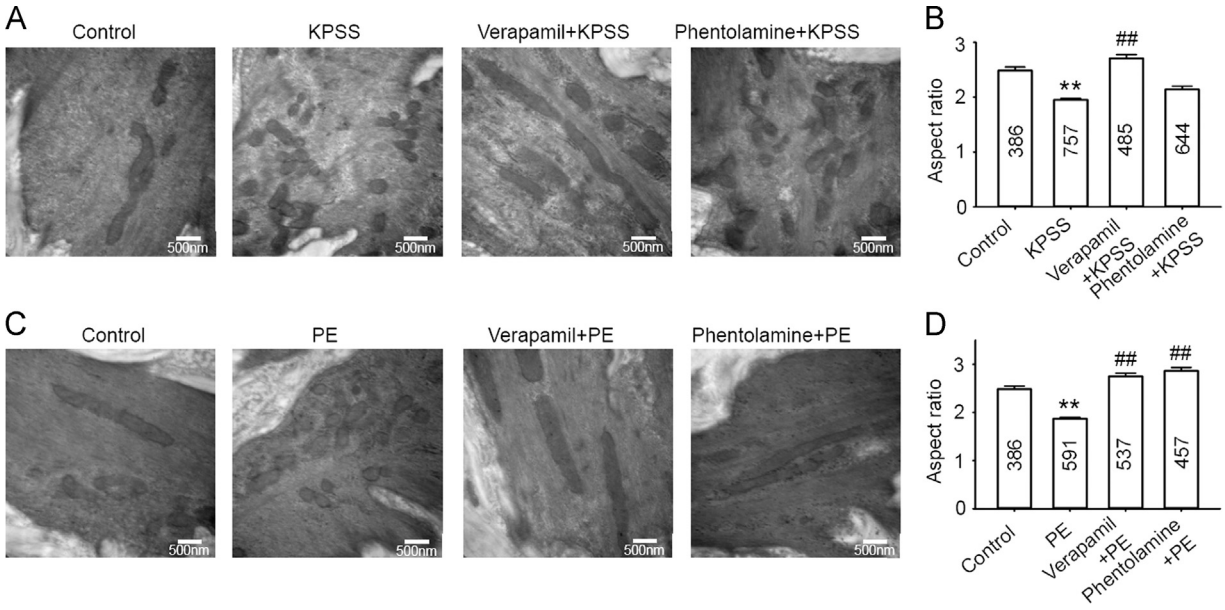



**Figure 5** Phentolamine pretreatment inhibited PE- but not KPSS-induced excessive mitochondrial fission of aortic smooth muscle cells and verapamil pretreatment inhibited both PE- and KPSS-induced excessive mitochondrial fission of aortic smooth muscle cells. (A) and (B) The typical images of transmission electron microscopy and the analyzed data showed that verapamil (5 μmol/L) but not phentolamine (1 μmol/L) pretreatment suppressed KPSS (60 mmol/L $K^+$)-induced excessive mitochondrial fission in smooth muscle cells of aorta. The numbers of mitochondria analyzed per group were shown in the bar. Aspect ratio, ratio between major and minor axes of an ellipse. $^{**}P<0.01$ *vs.* control; $P<0.01$ *vs.* KPSS, $n=6$. (C) and (D) The typical images of transmission electron microscopy and the analyzed data showed that verapamil (5 μmol/L) and phentolamine (1 μmol/L) pretreatments inhibited PE (1 μmol/L)-evoked excessive mitochondrial fission in smooth muscle cells of aorta. The numbers of mitochondria analyzed per group were shown in the bar. Aspect ratio, ratio between major and minor axes of an ellipse. $^{**}P<0.01$ *vs.* control; $P<0.01$ *vs.* PE, $n=6$.

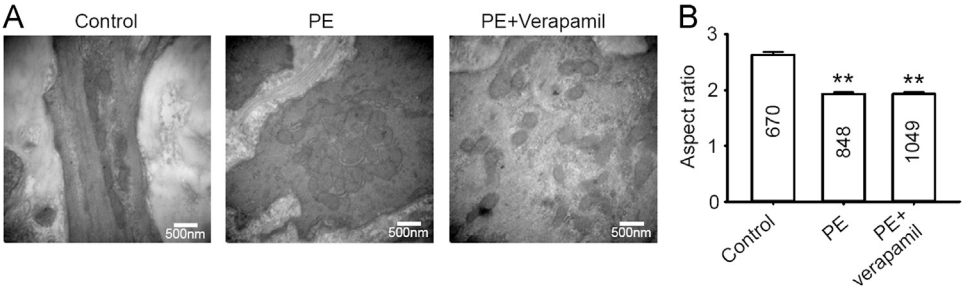



**Figure 6** Verapamil application after PE treatment had no effect on PE-induced excessive mitochondrial fission in smooth muscle cells of aorta. (A) The typical images of TEM. (B) The quantity analysis of mitochondrial fission. The numbers of mitochondria analyzed per group were shown in the bar. Aspect ratio, ratio between major and minor axes of an ellipse. $^{**}P<0.01$ *vs.* control.

inhibiting $Ca^{2+}$ channel. Thus, pre-treatment with verapamil, which reduced the store of intracellular $Ca^{2+}$, could weaken the effect of $Ca^{2+}$ release from sarcoplasmic reticulum induced by PE in smooth muscle cells in aorta, thereby antagonizing constriction of the aorta. A previous study reported that verapamil pretreatment reduced the rise of intracellular $Ca^{2+}$ induced by $PI(3,5)P_2$ in isolated aortic smooth muscle cells and accompanied by reductions in $PI(3,5)P_2$-induced constriction[19]. Such findings are in accord with the present findings.

### *3.4. The effect of pretreatment of phentolamine or verapamil on PE- or KPSS-induced excessive mitochondrial fission of aortic smooth muscle cells*

Since we postulated that vasoconstriction is coupled with mitochondrial fission in vascular smooth muscle cells[17], we used TEM to characterize mitochondrial morphology of smooth muscle cell in aorta treated with verapamil or phentolamine, followed by treatment of PE or KPSS. As shown in Fig. 5A and C, verapamil (but not phentolamine) pretreatment inhibited KPSS-induced excessive mitochondrial fission in smooth muscle cells of aorta. Phentolamine pretreatment inhibited PE-induced excessive mitochondrial fission in smooth muscle cells of aorta. Moreover, verapamil pretreatment prevented both PE- and KPSS-induced excessive mitochondrial fission of aortic smooth muscle cells. The statistical results are shown in Fig. 5B and D. The findings that verapamil pretreatment prevented both PE- and KPSS-induced increases in mitochondrial fission of smooth muscle cells are in accord with data showing that verapamil pretreatment inhibited both PE- and KPSS-induced vasoconstriction.

Our previous work demonstrated that the initial increase of cytosolic $[Ca^{2+}]_i$ triggered mitochondrial fission in vascular smooth muscle cells[17]. Verapamil inhibited KPSS-induced increases in cytosolic $[Ca^{2+}]_i$ through blocking L-type $Ca^{2+}$ channels, and phentolamine inhibited PE-induced increase of cytosolic $[Ca^{2+}]_i$ through blockade of $\alpha_1$-adrenergic receptor. As discussed above, the primary origin of intracellular $Ca^{2+}$ comes from extracellular $Ca^{2+}$ *via* $Ca^{2+}$ channels in vascular smooth muscle cells; verapamil pretreatment reduces the storage of intracellular $Ca^{2+}$ *via* inhibiting $Ca^{2+}$ channel, so it will weaken the effect of $Ca^{2+}$ release from sarcoplasmic reticulum induced by PE, thereby inhibiting PE-induced increase of cytosolic $[Ca^{2+}]_i$. These results further demonstrate that arterial relaxation is coupled to inhibition of mitochondrial fission in arterial smooth muscle cells.

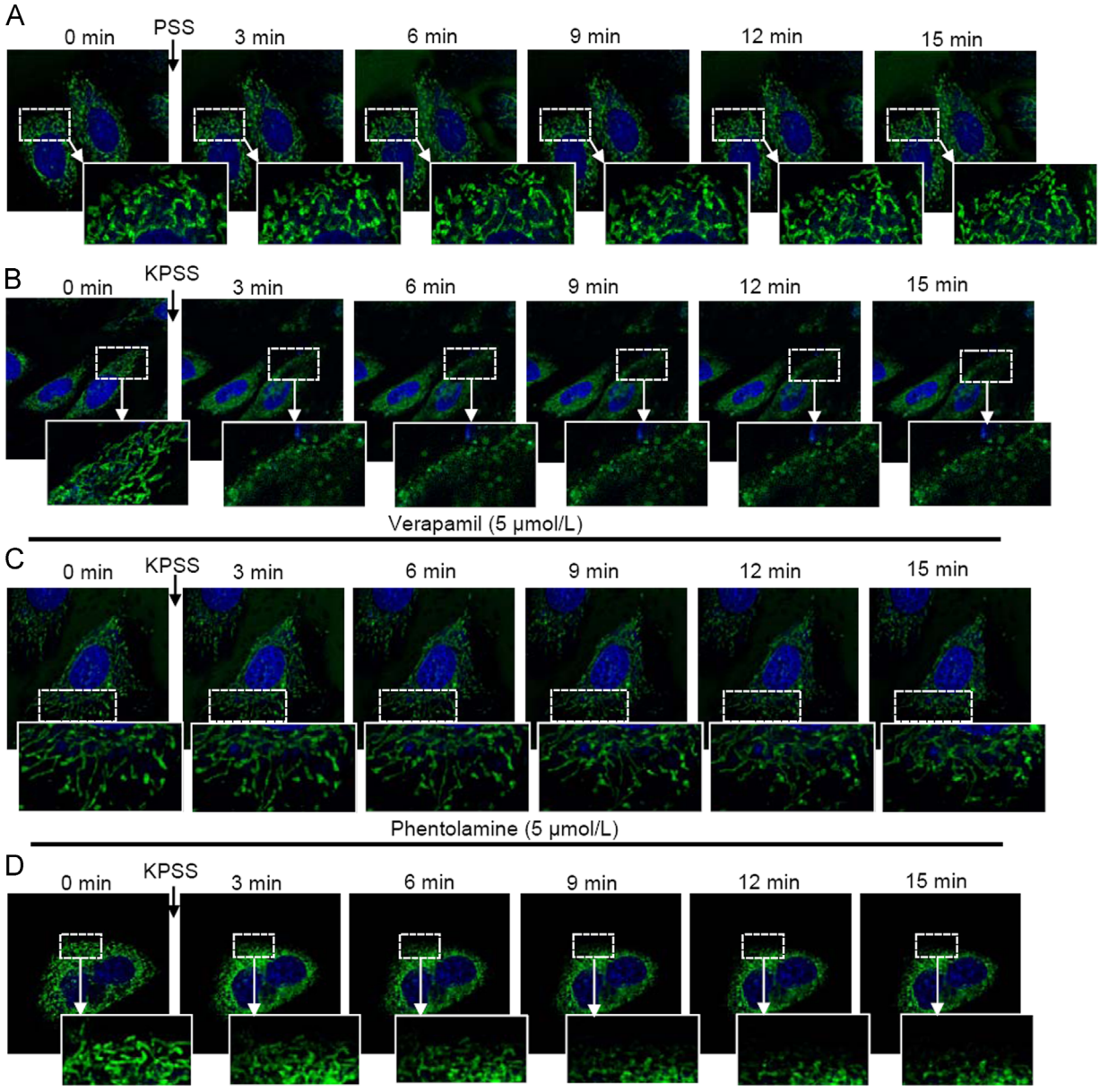



**Figure 7** Verapamil inhibited KPSS-induced excessive mitochondrial fission in cultured vascular smooth muscle cells (A10) accessed by laser confocal microscopy. The mitochondria of cultured vascular smooth muscle cells were stained with mitochondria-specific probe mitoTracker Green. The typical time-lapse images of vascular smooth muscle cells exposed to normal PSS were revealed in (A) and to KPSS (50 mmol/L $K^+$) were revealed in (B). The enlarged images of the framed area exhibited clear mitochondria fragmentation after treatment with KPSS (50 mmol/L $K^+$). (C) The time-lapse images of vascular smooth muscle cells pretreated with verapamil (5 μmol/L) for 30 min, and then exposed to KPSS (50 mmol/L $K^+$). The enlarged images of the framed area revealed that the integrity of mitochondria was not affected by KPSS (50 mmol/L $K^+$) in the presence of verapamil (5 μmol/L). (D) The time-lapse images of vascular smooth muscle cell pre-treated with phentolamine (5 μmol/L) for 30 min, and then exposed to KPSS (50 mmol/L $K^+$). The enlarged images of the framed area showed clear mitochondria fragmentation after treatment with KPSS (50 mmol/L $K^+$) in the presence of phentolamine (5 μmol/L).

### 3.5. *Verapamil application after PE treatment has no effect on PE-induced excessive mitochondrial fission*

Verapamil did not show vasorelaxant effects in aorta which was pre-contracted with PE (Fig. 1A). We then characterized mitochondrial morphology in smooth muscle cells from aorta in this state. As shown in Fig. 6, the mitochondrial fission of smooth muscle cells in aorta was increased after PE treatment. Verapamil did not inhibit this excessive mitochondrial fission in smooth muscle cells of PE-treated aorta. These results indicate that verapamil showed no inhibitory effect on mitochondrial fission when PE had induced intracellular $Ca^{2+}$ release and excessive mitochondrial fission. However, verapamil pretreatment reduced the storage of intracellular $Ca^{2+}$ in vascular smooth muscle cells, thereby inhibiting PE-induced vasoconstriction and PE-induced excessive mitochondrial fission.

### 3.6. *Verapamil inhibits KPSS-induced excessive mitochondrial fission of cultured vascular smooth muscle cells (A10)*

Mitochondrial fission dynamics were observed in the live cells by use of real-time confocal microscopy with mito-Tracker staining. As shown in Fig. 7, KPSS treatment induced mitochondrial fragmentation in cultured vascular smooth muscle cells (A10). Verapamil but not phentolamine inhibited KPSS-induced excessive mitochondrial fission. Nevertheless, phentolamine had no inhibitory effect on KPSS-induced excessive mitochondrial fission. Our previous study found that vascular smooth muscle cells lost sensitivity to PE after culturing[17], which even occurred in primary arterial smooth muscle cells[20]. Therefore, we did not use phentolamine to treat the cultured vascular smooth muscle cells to observe the effects of phentolamine on PE-induced excessive mitochondrial fission.

## 4. Conclusions

Based on our previous work, we used other two typical vasorelaxants, verapamil and phentolamine, to further prove the coupling between arterial constriction and mitochondrial fission in rat aorta. The present results demonstrate that arterial relaxation was completely congruent with the inhibition of mitochondrial fission in arterial smooth muscle cells. Together with our previous results[17], these findings show that studies with six different types of drugs (mdivi-1, dynasore, Y27632, nitroglycerin, verapamil and phentolamine) confirm the tight coupling between arterial constriction and mitochondrial fission in vascular smooth muscle cells. We suggest the existence of a novel physiological process "mitochondrial fission–contraction coupling" in arterial smooth muscle cells. Based on this hypothesis, pharmacological targeting of mitochondrial fission could be a novel approach to dilate arteries and lower blood pressure.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81373406 and 81421063).

## References

1. Hall AR, Burke N, Dongworth RK, Hausenloy DJ. Mitochondrial fusion and fission proteins: novel therapeutic targets for combating cardiovascular disease. *Br J Pharmacol* 2014;**171**:1890–906.
2. Li G, Jia ZQ, Cao Y, Wang YS, Li HT, Zhang ZY, et al. Mitochondrial division inhibitor 1 ameliorates mitochondrial injury, apoptosis, and motor dysfunction after acute spinal cord injury in rats. *Neurochem Res* 2015;**40**:1379–92.
3. Zhang CS, Lin SC. AMPK promotes autophagy by facilitating mitochondrial fission. *Cell Metab* 2016;**23**:399–401.
4. Sheng ZH, Cai Q. Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. *Nat Rev Neurosci* 2012;**13**:77–93.
5. Kasahara A, Scorrano L. Mitochondria: from cell death executioners to regulators of cell differentiation. *Trends Cell Biol* 2014;**24**:761–70.
6. Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Masuda K, et al. Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. *Nat Cell Biol* 2009;**11**:958–66.
7. Schrepfer E, Scorrano L. Mitofusins, from mitochondria to metabolism. *Mol Cell* 2016;**61**:683–94.
8. Yoon Y, Galloway CA, Jhun BS, Yu TZ. Mitochondrial dynamics in diabetes. *Antioxid Redox Signal* 2011;**14**:439–57.
9. Pennanen C, Parra V, López-Crisosto C, Morales PE, Del Campo A, Gutierrez T, et al. Mitochondrial fission is required for cardiomyocyte hypertrophy mediated by a $Ca^{2+}$-calcineurin signaling pathway. *J Cell Sci* 2014;**127**:2659–71.
10. Zepeda R, Kuzmicic J, Parra V, Troncoso R, Pennanen C, Riquelme JA, et al. Drp1 loss-of-function reduces cardiomyocyte oxygen dependence protecting the heart from ischemia-reperfusion injury. *J Cardiovasc Pharmacol* 2014;**63**:477–87.
11. Marín-García J, Akhmedov AT. Mitochondrial dynamics and cell death in heart failure. *Heart Fail Rev* 2016;**21**:123–36.
12. Cho B, Choi SY, Cho HM, Kim HJ, Sun W. Physiological and pathological significance of dynamin-related protein 1 (Drp1)-dependent mitochondrial fission in the nervous system. *Exp Neurobiol* 2013;**22**:149–57.
13. Itoh K, Nakamura K, Iijima M, Sesaki H. Mitochondrial dynamics in neurodegeneration. *Trends Cell Biol* 2013;**23**:64–71.
14. Marsboom G, Toth PT, Ryan JJ, Hong Z, Wu X, Fang YH, et al. Dynamin-related protein 1–mediated mitochondrial mitotic fission permits hyperproliferation of vascular smooth muscle cells and offers a novel therapeutic target in pulmonary hypertension. *Circ Res* 2012;**110**:1484–97.
15. Ryan J, Dasgupta A, Huston J, Chen KH, Archer SL. Mitochondrial dynamics in pulmonary arterial hypertension. *J Mol Med (Berl)* 2015;**93**:229–42.
16. Wang L, Yu T, Lee H, O'Brien DK, Sesaki H, Yoon Y. Decreasing mitochondrial fission diminishes vascular smooth muscle cell migration and ameliorates intimal hyperplasia. *Cardiovasc Res* 2015;**106**:272–83.
17. Liu MY, Jin J, Li SL, Yan J, Zhen CL, Gao JL, et al. Mitochondrial fission of smooth muscle cells is involved in artery constriction. *Hypertension* 2016;**68**:1245–54.
18. Zhang YQ, Shen X, Xiao XL, Liu MY, Li SL, Yan J, et al. Mitochondrial uncoupler carbonyl cyanide *m*-chlorophenylhydrazone induces vasorelaxation without involving $K_{ATP}$ channel activation in smooth muscle cells of arteries. *Br J Pharmacol* 2016;**173**:3145–58.
19. Silswal N, Parelkar NK, Wacker MJ, Brotto M, Andresen J. Phosphatidylinositol 3,5-bisphosphate increases intracellular free $Ca^{2+}$ in arterial smooth muscle cells and elicits vasocontraction. *Am J Physiol Heart Circ Physiol* 2011;**300**:H2016–26.
20. Fan LL, Ren S, Zhou H, Wang Y, Xu PX, He JQ, et al. $\alpha_{1D}$-Adrenergic receptor insensitivity is associated with alterations in its expression and distribution in cultured vascular myocytes. *Acta Pharmacol Sin* 2009;**30**:1585–93.