Berberine via suppression of transient receptor potential vanilloid 4 channel improves vascular stiffness in mice

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Abstract

Berberine, as an alkaloid found in many Chinese herbs, improves vascular functions in patients with cardiovascular diseases. We determined the effects of berberine in hypertension and vascular ageing, and elucidated the underlying mechanisms. In isolated aortas, berberine dose-dependently elicited aortic relaxation. In cultured cells, berberine induced the relaxation of vascular smooth muscle cells (VSMCs). Overexpression of transient receptor potential vanilloid 4 (TRPV4) channel by genetic approaches abolished the berberine-induced reduction in intracellular Ca²⁺ concentration in VSMCs and attenuated berberine-elicited vessel dilation in mice aortas. In deoxycorticosterone acetate (DOCA)-induced hypertensive model, treatment of mice with berberine or RN-1734, a pharmacological inhibitor of TRPV4, significantly decreased systemic blood pressure (BP) in control mice or mice infected with an adenovirus vector. However, berberine-induced effects of lowering BP were reversed by overexpressing TRPV4 in mice by infecting with adenovirus. Furthermore, long-term administration of berberine decreased mean BP and pulse BP, increased artery response to vasodilator and reduced vascular collagen content in aged mice deficient in apolipoprotein E (Apoe-KO), but not in Apoe-KO old mice with lentivirus-mediated overexpression of TRPV4 channel. In conclusion, berberine induces direct vasorelaxation to lower BP and reduces vascular stiffness in aged mice through suppression of TRPV4.

Keywords: berberine ● transient receptor potential vanilloid 4 ● vascular smooth muscle cells ● blood pressure ● vascular stiffness ● ageing

Introduction

Berberine, an isoquinoline alkaloid originally isolated from the Chinese herb Coptis chinensis, is an anti-microbial drug routinely prescribed for the treatment of diarrhoea in many Asian countries [1]. In this form it is reported to exert anti-fungal, anti-bacterial/viral and anti-oncogenic effects, as well as a beneficial effect on diabetes, atherosclerosis and hyperlipidemia [2, 3]. Although vasorelaxant effects of berberine have been observed in different animal models [4], the underlying mechanism and the effects of berberine on hypertension and vascular stiffness, which are associated with elevated vascular tone, are poorly understood.

Vascular smooth muscle cells (VSMCs) contraction is predominantly regulated by myosin light chain (MLC), which is determined by increased intracellular Ca²⁺ ((Ca²⁺)i) concentration through calmodulin (CaM)-dependent MLC kinase, resulting in phosphorylation of MLC at serine 19 and consequent contraction [5]. The Ca²⁺ influx is a common mechanism of transient increase in the cytoplasmic free Ca²⁺ concentration triggered by cell depolarization [6]. This form of Ca²⁺ signalling activates essential cellular processes including cardiac contraction, regulation of a smooth muscle tone, etc. Whether and how berberine reduces Ca²⁺ influx in VSMCs still remains unknown.

Transient receptor potential vanilloid 4 (TRPV4) is a Ca²⁺-permeable cation channel, originally identified as a transducer of hypotonic stimuli [7]. TRPV4 can be activated by various physical and chemical stimuli. Studies have reported that berberine produced inhibitory effects on Ca²⁺ channels [8]. Thus, we suggested that berberine via suppression of TRPV4 might lower blood pressure (BP) by inhibiting vessel contraction or eliciting vasorelaxation. Our data in this study suggest that berberine induces endothelium-independent relaxations in VSMCs to lower BP and to delay vascular stiffness by suppressing TRPV4 and the associated Ca²⁺ signalling in mice.
Materials and methods

Animals

Wild-type (WT, C57B16) mice and gene knockout of AMP-activated protein kinase (AMPKα1-KO), AMPKα2 (AMPKα2-KO), endothelial nitric oxide synthase (eNOS-KO), and apolipoprotein E (Apoe-KO) mice, 8–12 weeks of age, 20–25 g, were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed in temperature-controlled cages with a 12-hr light-dark cycle and given free access to water chows. The animal protocol was reviewed and approved by the University of Shandong University, Institute of Animal Care and Use Committee and the local ethics committee, in accordance with the Helsinki Declaration.

Measurement of tension development in aortic rings

In vivo or ex vivo organ chamber study was performed as described previously [9–12]. In short, descending aorta was cut into rings (3–4 mm in length) and suspended and mounted to organ chamber filled with Kreb’s buffer, gassed with 95% O2 plus 5% CO2. The contractile response was elicited by phenylephrine (PE), U46619 or KCl. Accumulative berberine, sodium nitroprusside (SNP) or phenolamine mesylate was added into the organ bath to induce vessel relaxation.

Blood pressure measurement

Blood pressure was determined by invasive left carotid catheter or radiotelemetry methods as described previously [13]. For invasive left carotid catheter, a catheter was inserted into the left common carotid artery. Blood was directed to a pressure transducer through the catheter, a catheter was inserted into the left common carotid artery. Radiotelemetry method of surgical procedure about insertion of radiotelemetry transmitter has been described above [13].

DOCA-salt hypertensive mice

DOCA-salt hypertension was created as previously described [14]. 150 mg/kg DOCA were implanted subcutaneously in mice given water containing 1.0% NaCl and 0.2% KCl.

A full description of materials and methods used, including generation of virus vector, cell culture, adenovirus infection to cells, measurement of [Ca2+]i concentration, western blot analysis, measurement of tension development in aortic rings, induction of hypertension by DOCA-salt in mice, BP measurement, picrosirius red staining, protocol for in vivo animal experiments in details and statistical analysis can be found in the Data S1.

Results

Berberine dose-dependently induces direct vessel relaxation in isolated mice aortas

We first determined the effects of berberine on vessel relaxation by organ chamber study. As shown in Figure 1A and B, aortic rings were pre-contracted by PE (1 μM), which induces vessel contraction through activation of G protein-coupled receptor (α-adrenoreceptor). When the contraction reached the peak and kept in stable state, accumulative berberine (0.1–100 μM) was added into an organ bath to induce vessel relaxation. Berberine, but not Dimethyl Sulphoxide (DMSO), started to relax aortic rings at 1 μM and completely reversed PE-induced contraction at 0.03 mM. In addition, berberine also dose-dependently induced vessel relaxation in aortic rings pre-contracted by U46619 at 30 nM (Fig. 1C and D), which is another activator of G protein-coupled receptor (thromboxane-receptor). Besides, the dose–response curves of berberine in PE- and U46619-contracted aortic rings were fitted by the Hill equation (Fig. 1B and D). The IC50 values of berberine on vessel relaxation were also calculated by fitting Hill equation to 6 individual data sets obtained from each aortic ring. The IC50s of berberine to induce relaxation in PE and U46619 are 8.93 ± 0.58 μM and 9.47 ± 0.62 μM, which are similar (P > 0.05), indicating berberine induces vessel relaxation in aortic arteries pre-contracted with multiple agonists.

Berberine suppresses the contractions of isolated mice aortas induced by agonists

We then investigated whether berberine suppressed vessel constriction in isolated mice aortic rings. Prior to induction of aortic contraction, isolated aortic rings from mice were incubated with berberine (10 μM) or DMSO for 60 min. As shown in Figure 1E and F, incubation of aortic rings with berberine but not DMSO significantly suppressed vessel contraction induced by PE and U46619, suggesting that berberine also has the function to attenuate vessel constriction.

Berberine-induced relaxation is endothelium-independent

To examine whether the vasodilation induced by berberine is mediated by endothelium, we detected the effects of berberine on vascular relaxation on aortic rings by removing the endothelium or by the incubation of aortas with eNOS inhibitor NG-Nitro-L-arginine Methyl Ester (L-NAME, 1 mM, 30 min.). As shown in Figure S1A and B, berberine dose-dependently induced vessel relaxation in endothelium-denuded aortas or L-NAME-pre-treated aortas, suggesting that berberine-induced relaxation is independent of endothelium (or eNOS). This viewpoint was further confirmed by...
using aortas from eNOS gene knockout mice (eNOS-KO). Similar to the removal of endothelium or L-NAME in aortas, deletion of eNOS did not alter berberine-induced vasorelaxation (Fig. S1A and B), providing further evidence of berberine-induced endothelium-independent relaxation.

AMPK is not involved in berberine-induced relaxation

In addition, berberine can activate AMPK in vascular wall cells [15, 16]. We determined whether berberine elicits relaxation on aortic rings if AMPK is inhibited. Unexpectedly, inhibition of AMPK by compound C (10 μM, 30 min.), which is a well-known AMPK inhibitor [17], did not abolish berberine-induced relaxation (Fig. S1C and D).

To exclude the possibility that these results were because of non-specific effects of compound C treatment, we repeated these experiments with mice aortas from AMPKα1 or α2 subunits gene knockout mice (AMPKα1-KO, AMPKα2-KO). As shown in Figure S1C and D, ablation of AMPKα1 or α2 did not change the effects of relaxation induced by berberine in WT mice aortas.

Berberine inhibits PE-induced MLC phosphorylations and contractions in cultured VSMCs

The contractile state of a VSMC induced by agonist depends on the phosphorylation of MLC at serine 19 [5, 18]. Thus, we assayed the effects of berberine on MLC phosphorylation by Western blot. As shown in Figure 2A, treatment of cultured VSMCs with PE (1 μM) for 30 min. dramatically increased MLC phosphorylation at serine 19 but not enhanced total MLC protein level. However, pre-treatment of VSMCs with berberine (1–100 μM, 5 min.) dose-dependently inhibited PE-induced MLC phosphorylation.

Further, we detected the alteration of cell morphology in berberine-pretreated PE-stimulated VSMCs in an in vitro cell culture model [19]. A 30-min. exposure of confluent VSMCs to PE (1 μM) resulted in spiderlike cell morphology (Fig. 2B). The contracted cells had narrow flaps around the round cell body. The perimeter and the area of contracted cells were decreased.
Berberine reduces intracellular Ca^{2+} signalling in VSMCs activated by agonists

We suggested that berberine via reduction of [Ca^{2+}]_i induces relaxation of VSMCs. To test this idea, we detected Ca^{2+} signalling by assaying [Ca^{2+}]_i level and CaM phosphorylation at serine 81, which represents its activity [20]. In Figure 2C, both PE and U46619 dramatically increased CaM serine 81 phosphorylation without altering total protein levels of CaM. Pre-incubation of VSMCs with berberine (10 μM) for 30 min. reduced CaM phosphorylation. As suggested, the [Ca^{2+}]_i level was significantly up-regulated by PE treatment assayed by measuring fluo-4/AM fluorescence (Fig. 2D and E). The PE-increased [Ca^{2+}]_i level is berberine-reversible.

Berberine-reduced VSMC contraction is VOCC-independent

A derivative of berberine, CPU86017 (as structured in Fig. S2), has been reported to perform as a blockade of voltage-operated calcium channel (VOCC) in myocardium [21]. Thus, we examined whether berberine also functions as an antagonist of VOCC to reduce [Ca^{2+}]_i levels by using KCl, which induces VSMC contraction, as a result of
membrane depolarization by causing Ca\textsuperscript{2+} entry through VOCC. As indicated in Figure S3A, 30-min. exposure of confluent VSMCs to KCl (60 mM) resulted in significant cell contractions. Unexpectedly, pre-incubation of VSMCs with berberine did not reverse the KCl-induced VSMC contractions.

We further confirmed the effects of berberine on KCl-induced vessel constriction in isolated mice aortas. Prior to induction of aortic contraction, isolated aortic rings from mice were incubated with berberine (10 \textmu M) or DMSO for 60 min. As shown in Figure S3B and C, incubation of aortic rings with either berberine or DMSO did not alter KCl-induced vessel contraction. Differently, berberine, which induced vasodilation in PE- or U46619-prechallenged vessel (Fig. 1A and B), failed to induce vasorelaxation in KCl-prechallenged aortic rings (Fig. S3D and E). Taking these data together, it suggests that berberine performs its function as a vasodilator, which is VOCC-independent.

Pharmacological activation of TRPV4 abolishes berberine-suppressed VSMC contractions in VSMCs

The TRPV4 channel is a regulator of intracellular Ca\textsuperscript{2+} in almost all cells which regulates vascular tone and BP [22–24]. Thus, we examined whether berberine functions as an antagonist of TRPV4 to reduce the [Ca\textsuperscript{2+}] level. We used the known TRPV4 agonists (4\textalpha-PDD, GSK1016790A) or antagonists (RN-1734) to activate or inhibit TRPV4, of which the chemical structures are shown in Figure S2. Incubation of VSMCs with berberine or RN-1734 significantly reversed the morphological alteration of cultured VSMCs from resting to contractile (Fig. S4A, c and d) or PE/U46619-induced contraction in aortic rings (Fig. 1E and F). However, berberine was unable to reverse PE-induced VSMC contractions in presence of 4\textalpha-PDD or GSK1016790A (Fig. S4A, e and f). Berberine derivative of CPU86017 also induced VSMCs relaxation if TRPV4 was not activated (Fig. S4A, g and h), indicating that TRPV4 might be a pharmacological target of berberine to suppress VSMC contractions.

Overexpression of TRPV4 abolishes berberine-induced reduction of intracellular Ca\textsuperscript{2+} concentration in VSMCs

We infected VSMCs with adenovirus containing TRPV4 cDNA to confirm whether berberine via suppression of TRPV4 induces VSMC relaxation. As shown in Figure S5A, 48-hr adenovirus infection
dramatically increased TRPV4 protein expression in cell membrane. PE dramatically increased \([\text{Ca}^{2+}]_i\) levels in VSMCs infected with both vector and TRPV4 as indicated by fluo-4/AM fluorescence (Fig. 3A and B). Although berberine still effectively reduced PE-induced enhancement of \([\text{Ca}^{2+}]_i\) levels in vector-infected VSMCs, it failed to suppress the up-regulation of \([\text{Ca}^{2+}]_i\) levels in PE-stimulated VSMCs when TRPV4 was overexpressed. Besides, overexpression of TRPV4 bypasses berberine-inhibited CaM and MLC phosphorylations (Fig. 3C) and consequent VSMC contraction (Fig. S4B). These results demonstrate that berberine via suppression of TRPV4 reduces \([\text{Ca}^{2+}]_i\), signalling to relax VSMCs.

**Berberine via suppression of TRPV4 inhibits vessel contraction and induces vasorelaxation in isolated mice aortas**

Next we detected the effects of overexpression of TRPV4 on berberine-reduced contraction on mice aortas. Mice were infected with adenovirus to overexpress TRPV4 in aortas. As shown in Figure 4A and B, incubation of aortic rings with berberine dramatically suppressed PE-induced contractions in aortas from mice infected with the vector. However, berberine did not inhibit PE-induced vasoconstriction in aortas from mice when TRPV4 was upregulated. Similar to suppression on vessel contraction, berberine elicited direct relaxation in PE/U46619-challenged aortic rings isolated from mice infected with vector (Fig. 4C–E). However, these effects of berberine on vasodilation were attenuated by up-regulation of TRPV4.

**Berberine lowers DOCA-salt-induced systemic hypertension in mice**

We next tested the effects of berberine on DOCA-salt-induced hypertension. Wild-type (C57B16) mice were implanted with DOCA-salt to establish hypertensive model. Systemic BP was measured by radiotelemetry method. As shown in Figure 5A and B, both systolic BP and diastolic BP were increased to the high level at the 10th day and were stable upto the 35th day after DOCA-salt treatment. Administration of
mice with berberine completely suppressed DOCA-salt-induced increases of systolic BP and diastolic BP. The effects of berberine on lowering BP were mimicked by a recognized TRPV4 antagonist, RN-1734, indicating that berberine may function as TRPV4 antagonist to lower salt-sensitive BP.

Overexpression of TRPV4 abolishes the berberine-induced effects of lowering BP in DOCA-salt-treated mice

To further confirm berberine via suppression TRPV4 decreases BP, we generated TRPV4-overexpressed mice by infecting WT mice with adenovirus (Fig. S5B). Similar to control C57B16 mice without any infections (Fig. 5A and B), berberine also produced significantly BP-lowering effects in mice infected with adenovirus containing vector when implanted with DOCA-salt (Fig. 5C and D). As expected, while TRPV4 was up-regulated by adenovirus infection in mice, the increased systolic BP and diastolic BP were not inhibited significantly by berberine (Fig. 5E and F). These data demonstrate that the TRPV4 is involved in berberine-induced effects on lowering BP.

Long-term administration of berberine, through suppression of TRPV4, decreases pulse BP in aged mice

To investigate whether berberine via suppression of TRPV4 improves vessel elasticity in old mice, we generated TRPV4 overexpressed mice by infecting high-fat-diet-fed Apoe-KO mice with lentivirus (Fig. S5C). As depicted in Figure 6A and B, 1-year administration of berberine in Apoe-KO mice fed with high-fat diet obviously decreased mean BP and pulse BP (131 ± 17 versus 114 ± 12 mmHg for mean BP, P < 0.05; 55 ± 7 versus 37 ± 4 mmHg for pulse BP, P < 0.05).
when mice were infected with lentivirus vector alone. However, long-term berberine administration did not lower mean BP and pulse BP (140 ± 23 versus 137 ± 18 mmHg for mean BP, P > 0.05; 56 ± 9 versus 51 ± 6 mmHg for pulse BP, P > 0.05) if mice were infected with lentivirus expressing TRPV4. These findings indicate that berberine delays vascular stiffness via suppression of TRPV4 in aged mice.

Up-regulation of TRPV4 inhibits artery response to vasodilators in berberine-treated aged mice

It is worthy of assaying bioactive response of artery to SNP and phentolamine, which are well-used drugs in hypertensive patients. As indicated in Figure 6C and D, SNP- or phentolamine-induced dose-dependent relaxation in abdominal aortic ring were improved dramatically in lentivirus-vector-infected mice with 1-year administration of berberine, compared with mice without berberine treatment. However, berberine did not improve vasorelaxation induced by SNP or phentolamine in mice infected with lentivirus expressing TRPV4. Collectively, it suggests that berberine improves vessel bioactivity in aged mice, which is mediated by inhibition of TRPV4.

Berberine via inhibition of TRPV4 reduces collagen contents in artery wall in aged mice

Pathologically, accumulative collagen deposition in artery wall is a character of vascular stiffness [25, 26]. We finally determined the collagen contents in artery by picrosirius red staining. Similarly, when TRPV4 was up-regulated in Apoe-KO mice, the collagen contents were not reduced significantly by berberine, but it was decreased in mice without overexpression of TRPV4 (Fig. 6E and F). These data demonstrate that the TRPV4 is also involved in berberine-mediated effects on collagen deposition in the artery wall of aged mice.
Discussion

In this study, we provide the first evidence that administration of berberine in vivo lowers high BP in DOCA-induced hypertensive mice and reduces vascular stiffness in aged Apoe-KO mice. Mechanically, these effects of berberine are attributable to suppression of TRPV4, decreased [Ca^{2+}]_{i} levels, decreased CaM/MLC activity and consequent relaxations in VSMC. In this way, berberine functions as a calcium channel blocker to decreases high BP and vascular hardness.

The major finding of this study is that berberine suppresses DOCA-induced hypertension and ageing-induced vascular stiffness. Physiologically, hypertension and vascular stiffness is related to vascular flexibility and compliance. Previous studies have reported that berberine or its derivative blocked Ca^{2+} influx as a blockade of L-type Ca^{2+} channel in myocardium to treat heart failure [8, 27]. In the present study, we further identified that TRPV4 as a novel target of berberine to regulate vascular tone through direct relaxation on VSMCs by suppressing Ca^{2+} entry. Accordingly, in vitro or in vivo overexpression of TRPV4 abolished reduction in [Ca^{2+}]_{i}, levels in berberine-treated cells and relaxation in isolated aortas from mice fed with berberine. Thus, we reason the exact mechanism of berberine blocking TRPV4 is possibly related to the bind of berberine to the pore core of TRPV4, which is the core of Ca^{2+} channel [28], to misconduct Ca^{2+} current (Fig. S6), similar to RN-1734 does. As a shortcoming of this study, we can only speculate berberine can directly target on TRPV4. Solid evidence should be provided to be sure this possibility.

In general, the function of TRPV4 on vascular endothelial cell in pulmonary artery was sure to induce endothelium-dependent relaxation, which is sensitive to high salt [22, 23, 29–31]. The functions of TRPV4 in other cells or other arteries are more controversial. In this study, we found that RN-1734, a TRPV4 antagonist, significantly inhibited PE/U46619-induced contraction in aortic rings (Fig. 1E and F), indicating that activation of TRPV4 was involved in the PE/ U46619-induced vessel contraction. For example, Earley et al. reported that TRPV4 activation via signalling coupling to ryanodine receptors and BKca channels causes vascular smooth muscle hyperpolarization and relaxation in rat cerebral artery [32], inconsistent with our observations. We thought that this discrepancy is because of the different arteries used in our and their studies. Moreover, it is unclear whether and how PE and U46619 can induce TRPV4 activation, which can both activate their specific receptors to induce contraction of VSMCs by increasing intracellular Ca^{2+} concentration [18]. TRPV4, as a Ca^{2+} permeable non-selective cationic channel, is activated by stimuli including physical, thermal and chemical stimuli, cell swelling, shear stress in particular cells. Importantly, intracellular Ca^{2+}, depending on the concentration, either potentiates or inhibits the TRPV4 channel activity [22, 28]. Thus, we speculate that PE and U46619 can induce TRPV4 activation through elevated intracellular Ca^{2+} concentration.

A major limitation of this study is that the range of berberine to induce vasorelaxation is 10–30 μM. Although other groups have also reported that berberine enhances the endothelium-dependent vasorelaxation and endothelium-independent VSMC dilatation or inhibits the endothelium-independent contraction induced by an agonist at similar concentration [33, 34], such concentration of berberine action should not be limited to the Ca^{2+}-permeable channel. Membrane penetration and diffusion rate should be analysed to determine the pharmaceutical kinetics of berberine in VSMC. Besides, it has been reported [35] that the K+ channel blockers significantly attenuated berberine-induced vasodilatation in the endothelium-denuded arteries, indicating the role of K+ channel in berberine-induced VSMC contraction. Further study should focus on the effects of K+ channel in VSMC.

In summary, we are the first to report that berberine may suppress TRPV4, decrease [Ca^{2+}]_{i}, levels and CaM/MLC activity, and induce consequent relaxation in VSMC (Fig. S7). This leads to the anti-hypertensive and anti-vascular ageing effects of berberine in mice. Our discovery of characterization of berberine as an antagonist of the TRPV4 channel would help in the elucidation of TRPV4 function and considered it as a clinical drug to target TRPV4 in treating the high-tension of vascular tone-related cardiovascular diseases, such as hypertension, vascular stiffness and stroke.

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Conflicts of interest

The authors confirm that there are no conflicts of interest.

Author contribution

J.W designed and conducted the experiments, and analysed data. T.G. and S.W.Y. partially performed experiments. Q.M.P. gave some suggestions to this article. S.X.W. designed and conducted the experiments, analysed data, convinced the project, wrote and revised the manuscript.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1 Supplementary Materials and Methods.
References

1. Affuso F, Mercurio V, Fazio V, et al. Cardiovascular and metabolic effects of Berberine. World J Cardiol. 2010; 2: 71–7.
2. Mokher-Dezfuli N, Saedinnia S, Gohari AR, et al. Phytochemistry and pharmacology of berberis species. Pharmacogn Rev. 2014; 8: 8–15.
3. Wang Q, Zhang M, Liang B, et al. Activation of AMP-activated protein kinase is required for berberine-induced reduction of atherosclerosis in mice: the role of uncoupling protein 2. PLoS ONE. 2011; 6: e25436.
4. Lau CW, Yao XQ, Chen ZY, et al. Cardiovascular actions of berberine. Cardiovasc Drug Rev. 2001; 19: 234–44.
5. Ratz PH, Berg KM, Urban NH, et al. Regulation of smooth muscle calcium sensitivity: KCl as a calcium-sensitizing stimulus. Am J Physiol Cell Physiol. 2005: 288: C769–83.
6. Wang J, Thio SS, Yang SS, et al. Splice variant specific modulation of CaV1.2 calcium channel by galectin-1 regulates arterial constriction. Circ Res. 2011; 109: 1290–8.
7. Ueda T, Shikano M, Kamiya T, et al. The TRPV4 channel is a novel regulator of intracellular Ca(2+) in human esophageal epithelial cells. Am J Physiol Gastrointest Liver Physiol. 2011; 301: G138–47.
8. Wang F, Zhou HY, Zhao G, et al. Inhibitory effects of berberine on ion channels of rat hepatocytes. World J Gastroenterol. 2004; 10: 2842–5.
9. Wang S, Liang B, Viollet B, et al. Inhibition of the AMP-activated protein kinase-alpha2 accentuates agonist-induced vascular smooth muscle contraction and high blood pressure in mice. Hypertension. 2011; 57: 1010–7.
10. Wang S, Peng Q, Zhang J, et al. Na+/H+ exchanger is required for hyperglycaemia-induced endothelial dysfunction via calcium-dependent calpain. Cardiovasc Res. 2008; 80: 255–62.
11. Shuang-Xi W, Li-Ying L, Hu M, et al. Na+/H+ exchanger inhibitor prevented endothelial dysfunction induced by high glucose. J Cardiovasc Pharmacol. 2005; 45: 586–90.
12. Wang SX, Xiong XM, Song T, et al. Protective effects of cariporide on endothelial dysfunction induced by high glucose. Acta Pharmacol Sin. 2005; 26: 329–33.
13. Liang B, Wang S, Wang Q, et al. Aberrant endoplasmic reticulum stress in vascular smooth muscle increases vascular contractility and blood pressure in mice deficient of AMP-activated protein kinase-alpha2 in vivo. Arterioscler Thromb Vasc Biol. 2013; 33: 595–604.
14. Du YH, Guan YY, Alp NJ, et al. Endothelium-specific GTP cyclohydrolase I overexpression attenuates blood pressure progression in salt-sensitive low-renin hypertension. Circulation. 2008; 117: 1045–54.
15. Han Y, Wang Q, Song P, et al. Redox regulation of the AMP-activated protein kinase. PLoS ONE. 2010; 5: e15420.
16. Liang KW, Yin SC, Ting CF, et al. Berberine inhibits platelet-derived growth factor-induced growth and migration partly through an AMPK-dependent pathway in vascular smooth muscle cells. Eur J Pharmacol. 2008; 590: 343–54.
17. Wang S, Zhang M, Liang B, et al. AMPKalpha2 deletion causes aberrant expression and activation of NAD(P)H oxidase and consequent endothelial dysfunction in vivo: role of 26S proteasomes. Circ Res. 2010; 106: 1117–28.
18. Somlyo AP, Somlyo AV. Ca(2+) sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. Physiol Rev. 2003; 83: 1325–58.
19. Nagy Z, Kolev K, Csonka E, et al. Contraction of human brain endothelial cells induced by thrombogenic and fibrinolytic factors: an in vitro cell culture model. Stroke. 1995; 26: 265–70.
20. Greif DM, Sacks DB, Michel T, Calmodulin phosphorylation and modulation of endothelial nitric oxide synthase catalysis. Proc Natl Acad Sci USA. 2004; 101: 1165–70.
21. Qi MY, Feng Y, Dai DZ, et al. CPU86017, a berberine derivative, attenuates cardiac failure by normalizing calcium leakage and downregulated phospholamban and exerting antioxidant activity. Acta Pharmacol Sin. 2010; 31: 165–74.
22. Firth AL, Remillard CV, Yuan JX. TRP channels in hypertension. Biochim Biophys Acta. 2007; 1772: 895–906.
23. Bagher P, Belezanski T, Kansui Y, et al. Low intravascular pressure activates endothelial cell TRPV4 channels, local Ca(2+) events, and IKCa channels, reducing arteriolar tone. Proc Natl Acad Sci USA. 2012; 109: 18174–9.
24. Filosa JA, Yao X, Rath G. TRPV4 and the regulation of vascular tone. J Cardiovasc Pharmacol. 2013; 61: 113–9.
25. Xu J, Shi GP. Vascular wall extracellular matrix proteins and vascular diseases. Biochim Biophys Acta. 2014; 1842: 2106–19.
26. O’Rourke MF, Mancia G. Arterial stiffness. J Hypertens. 1999; 17: 1–4.
27. Dai DZ, Hu HJ, Zhao J, et al. Blockade of L-type calcium channel in myocardium and calcium-induced contractions of vascular smooth muscle by CPU 86017. Acta Pharmacol Sinica. 2004; 25: 416–23.
28. Everaerts W, Nilius B, Owsianik G. The vanilloid transient receptor potential channel TRPV4: from structure to disease. Prog Biochem Mol Biol. 2010; 103: 2–17.
29. Sukumaran SV, Singh TU, Parida S, et al. TRPV4 channel activation leads to endothelium-dependent relaxation mediated by nitric oxide and endothelium-derived hyperpolarizing factor in rat pulmonary artery. Pharmaco Res. 2013; 78: 18–27.
30. Zhang DX, Mendoza SA, Bubolz AH, et al. Transient receptor potential vanilloid type 4-deficient mice exhibit impaired endothelium-dependent relaxation induced by acetylcholine in vivo and in vitro. Hypertension. 2009; 53: 532–6.
31. Ma X, Da J, Zhang P, et al. Functional role of TRPV4-KCNa2.3 signaling in vascular endothelial cells in normal and streptozotocin-induced diabetic rats. Hypertension. 2013; 62: 134–9.
32. Earley S, Hoppner TJ, Nelson MT, et al. TRPV4 forms a novel Ca(2+) signaling complex with ryabondine receptors and BKCa channels. Circ Res. 2005; 97: 1270–9.
33. Yang W, Huang Y, Lam KS, et al. Berberine prevents hyperglycemia-induced endothelial injury and enhances vasodilation via adenosine monophosphate-activated protein kinase and endothelial nitric oxide synthase. Cardiovasc Res. 2009; 82: 484–92.
34. Ko WH, Yao XQ, Lau CW, et al. Vasorelaxant and antiproliferative effects of berberine. Eur J Pharmacol. 2000; 399: 187–96.
35. Wang YC, Zheng YM, Zhou XB. Inhibitory effects of berberine on ATP-sensitive K+ channels in cardiac myocytes. Eur J Pharmacol. 1996; 316: 307–15.