

Berberine reduced blood pressure and improved vasodilation in diabetic rats

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Abstract

Hyperglycemia and hypertension are considered to be the two leading risk factors for vascular disease in diabetic patients. However, few pharmacologic agents could provide a combinational therapy for controlling hyperglycemia and hypertension at the same time in diabetes. The objectives of this study are to investigate whether berberine treatment could directly reduce blood pressure and identify the molecular mechanism underlying the vascular protection of berberine in diabetic rats. Berberine was intragastrically administered with different dosages of 50, 100 and 200 mg/kg/day to diabetic rats for 8 weeks since the injection of streptozotocin. The endothelium-dependent/-independent relaxation in middle cerebral arteries was investigated. The activity of large-conductance Ca²⁺-activated K⁺ channel (BK_{Ca}) was investigated by recording whole-cell currents, analyzing single-channel activities and assessing the expressions of α - and β 1-subunit at protein or mRNA levels. Results of the study suggest that chronic administration of 100 mg/kg/day berberine not only lowered blood glucose but also reduced blood pressure and improved vasodilation in diabetic rats. Furthermore, berberine markedly increased the function and expression of BK_{Ca} β 1-subunit in cerebral vascular smooth muscle cells (VSMCs) isolated from diabetic rats or when exposed to hyperglycemia condition. The present study provided initial evidences that berberine reduced blood pressure and improved vasodilation in diabetic rats by activation of BK_{Ca} channel in VSMCs, which suggested that berberine might provide a combinational therapy for controlling hyperglycemia and blood pressure in diabetes. Furthermore, our work indicated that activation of BK_{Ca} channel might be the underlying mechanism responsible for the vascular protection of berberine in diabetes.

Key Words

- ▶ berberine
- ▶ relaxation
- ▶ vascular smooth muscle cells (VSMCs)
- ▶ large-conductance calcium-activated K⁺ channel (BK_{Ca})

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Introduction

Vascular disease is a leading cause of morbidity and mortality in diabetic patients (Sena *et al.* 2013, Tousoulis *et al.* 2013). Although many risk factors could accelerate the progression of diabetic vascular disease,

hyperglycemia and hypertension are found to coexist frequently and considered to be the two leading risk factors (Cheung & Li 2012). Clinical trials and animal studies have indicated that certain oral hypoglycemic

drugs, such as metformin and gliclazide, are beneficial for the control of cardiovascular remodeling in diabetic patients (Landman *et al.* 2014, Ladeiras-Lopes *et al.* 2015). In addition, some antihypertensive agents, such as calcium channel blockers or angiotensin-converting enzyme inhibitors (ACEIs), are also effective to alleviate cardiovascular remodeling in diabetes (Tillin *et al.* 2011, Ettehad *et al.* 2016). However, few pharmacologic agents could provide a combinational therapy for controlling hyperglycemia and hypertension at the same time in diabetic patients at present (Mukherjee *et al.* 2015).

Berberine is extracted from *Hydrastis canadensis*, *Berberis aquifolium* and *Chinese goldthread* (Chang *et al.* 2014). Berberine has been reported to have anti-bacterial/anti-parasitic properties and anti-oxidative/anti-inflammatory activities (Ni *et al.* 2015). Recently, human and animal studies indicated that berberine also had a hypoglycemic activity (Zhang & Chen 2012) and an extra-beneficial protection for cardiovascular system in hypertension or diabetes (Wang *et al.* 2012, Li *et al.* 2014, Ni *et al.* 2015). Our previous work observed that berberine alleviated cerebral arterial contractility in streptozotocin (STZ)-induced diabetic rats by regulating the intracellular Ca^{2+} handling of smooth muscle cells (Ma *et al.* 2016). In addition, berberine has been reported to improve vascular endothelial function in humans (Cheng *et al.* 2013) and show the protective effects for hypertensive patients with type 2 DM as the adjuvant therapy (Dai *et al.* 2015). However, it remains unknown whether or not berberine treatment could directly reduce blood pressure in diabetes. Furthermore, it is not clear what is the molecular mechanism underlying the vascular protection of berberine treatment in diabetes.

Vascular myogenic tone is one of the important factors to determine blood pressure. Increase in intracellular Ca^{2+} is the primary trigger to activate myosin light chain kinase (MLCK), which phosphorylates myosin light chains (MLCs), activates myosin ATPase and leads to vascular contraction. Conversely, vascular relaxation is mediated by activation of myosin light chain phosphatase (MLCP), which dephosphorylates myosin light chains to cause relaxation. Large-conductance Ca^{2+} -activated K^+ (BK_{Ca}) channels are widely expressed in vascular smooth muscle cells (VSMCs). When the extracellular Ca^{2+} enters through voltage-dependent Ca^{2+} channels (VDCCs), the ryanodine receptors (RyRs) in sarcoplasmic reticulum (SR) are activated and then produce the ' Ca^{2+} -induced Ca^{2+} release (CICR)'. This CICR activates nearby BK_{Ca} channels and induces membrane hyperpolarization, which then

leads to vascular relaxation (Krishnamoorthy-Natarajan & Koide 2016). It has been reported that increased vascular tone in diabetes may arise from the impaired function of BK_{Ca} channel (Nystoriak *et al.* 2014). In addition, it has been demonstrated that chronic hyperglycemia-induced depression of BK_{Ca} channels may be mechanistically linked to the protein kinase C (PKC)-mediated phosphorylation (Vetri *et al.* 2012). All these results suggested that BK_{Ca} channel is likely to be one of the most important therapeutic targets for diabetic vascular complications.

The purpose of the present study was twofold: (1) to investigate the effects of berberine treatment on blood pressure and vasodilation in STZ-induced diabetic rats; and (2) to examine the effects of berberine on BK_{Ca} channels in diabetic rats or when exposed to hyperglycemic condition by recording whole-cell currents, analyzing single-channel activities and assessing the expressions of α - and β 1-subunit at protein or mRNA levels. Taken together, the present study provided initial evidence for the first time that berberine contributes to reducing blood pressure and improving the vasodilation in diabetic rats by the direct activation of BK_{Ca} channel in VSMCs, which suggested that berberine treatment might provide a combinational therapy for lowering blood glucose and reducing blood pressure. Furthermore, our work indicated that activation of BK_{Ca} channel in VSMCs might be the underlying mechanism responsible for the vascular protection of berberine in diabetes.

Materials and methods

All animal procedures were in adherence with the Animals in Research: Reporting *In Vivo* Experiments (ARRIVE) guidelines (Kilkenny *et al.* 2010) and the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Unless otherwise stated, all reagents were obtained from Sigma Chemical Company.

Animal model

Male Sprague–Dawley rats (~190 g) were purchased from Medical Laboratory Animal Center of Xi'an Jiaotong University. According to our previous description (Ma *et al.* 2016), a high-fat diet plus low-dose injections of streptozotocin (STZ) were used to establish the diabetic rat. The high-fat diet consisted of 70% standard laboratory chow, 5% yolk powder, 10% lard and 15% carbohydrate. The control rats were given the regular

diet. Following 4 weeks of dietary intervention, rats were injected intraperitoneally with 30 mg/kg STZ, which was freshly dissolved in 0.1 M sodium citrate buffer (pH 4.5–5.0), consecutively twice in 2 days. In contrast, the control rats were injected intraperitoneally with vehicle citrate buffer in a dose volume of 1 mL/kg. Experiment I, Experiment II and Experiment III were divided into 4 groups: control rats (CON), control rats administered with 50, 100 and 200 mg/kg/day berberine chloride (CON+berberine), diabetic rats and diabetic rats administered with 50, 100 and 200 mg/kg/day berberine chloride (Diabetic+berberine), respectively (Wu *et al.* 2012, Moghaddam *et al.* 2013). Afterward, the diabetic and control rats were fed on the high-fat diet and the regular diet for another 8 weeks, respectively. Berberine chloride was dissolved and then intragastrically administered once daily for 8 weeks. The other groups received equal volume of vehicles. Blood pressure in mammalian animals has a circadian pattern characterized by a low period during sleep and a high plateau period in the awake state. For rats, the BP decreases in the light time (a low period) and increases in the dark time (a high plateau period) (Lim *et al.* 2016). In the present study, the systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were taken by the tail-cuff method at 10:00, 16:00 in the day time and 22:00, 04:00, respectively. Every animal was measured for BP consecutively in 3 days (Xie 2005).

Examination of vascular relaxation

As previously described (Zhang *et al.* 2008, Lin *et al.* 2009), the middle cerebral artery was isolated and removed to the chilled physiological salt solution (PSS) which contained (in mM) 119 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, 2.5 CaCl₂, 5.5 glucose and 0.026 EDTA, equilibrated with 21% O₂, 5% CO₂ and 74% N₂ at pH 7.4 adjusted with NaOH. After cannulation, the vessel chamber was placed into the Pressure Myograph System P110 (DMT, Denmark). Following an equilibration for 1 h at 37°C and a pressure of 50 mmHg, vascular contraction was induced by 60 mM KCl and then the superfusion of acetylcholine (ACh; 10⁻¹⁰–10⁻⁴ M) and sodium nitroprusside (SNP; 10⁻¹⁰–10⁻⁴ M) induced the relaxation, respectively. We tested the vascular dilation in the superfusing solution containing ACh or SNP from the low concentration (10⁻¹⁰) to high concentration (10⁻⁴ M) one by one consecutively. For example, when the vascular diameter reached a stable condition and was kept for 3 min under low concentration (10⁻⁷ M ACh), the

next concentration (10⁻⁶ M ACh) was then added to the superfusing solution for the next investigation of vascular diameter according to our previous report (Zhang *et al.* 2008, Lin *et al.* 2009). Vasodilation was represented by the percentage of luminal diameter relative to the constriction diameter induced by 60 mM KCl.

Electrophysiological measurements

Single VSMC was isolated as previously described (Xie *et al.* 2010). Briefly, brain tissues were transferred to the chilled PSS containing (in mM) 137 NaCl, 5.6 KCl, 1 MgCl₂, 0.42 Na₂HPO₄, 0.44 NaH₂PO₄, 4.2 NaHCO₃ and 10 HEPES, equilibrated with 95% O₂ and 5% CO₂ at pH adjusted to 7.4 with NaOH. The cerebral arteries were digested for 18 min at 37°C with 4 mg/mL papain (Biochrom, Berlin, Germany), 2 mg/mL dithioerythritol (Amresco, St Louis, Missouri, USA), 1 mg/mL bovine serum albumin (BSA) and 5 mM taurine in buffering solution. Whole-cell and single-channel of BK_{Ca} currents were recorded according to our previous reports (Ma *et al.* 2010, Xie *et al.* 2010, Chang *et al.* 2011). The extracellular (bath) solution contained (in mM) 135 NaCl, 5 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 HEPES, 10 glucose, 5 4-AP, equilibrated with 95% O₂ and 5% CO₂ at pH 7.4 adjusted by NaOH. The internal (pipette) solution contained (in mM) 50 KCl, 70 K-Asp, 8 NaCl, 2 MgCl₂, 1 Na₂ATP, 0.5 GTP, 10 HEPES, 1 CaCl₂, 2 EGTA equilibrated with 95% O₂ and 5% CO₂ at pH 7.2 titrated with KOH. BK_{Ca} single-channel currents were recorded in cell-attached membrane patches. The pipette (external) solution contained (in mM) 40 K-Asp, 100 KCl, 1.0 CaCl₂, 10 HEPES equilibrated with 95% O₂ and 5% CO₂ at pH 7.4 titrated with KOH. The bath solution contained 100 K-Asp, 40 KCl, 10 HEPES, 3.6 CaCl₂ equilibrated with 95% O₂ and 5% CO₂ at pH 7.4 titrated with KOH.

Evaluation of BK_{Ca} protein expression by Western blotting

The protein samples of cerebral arteries were prepared as previously described (Xie *et al.* 2010). Cerebral arteries were homogenized with tissue protein extraction reagent (T-PER, Pierce) and protease inhibitor (Halt, Pierce). The protein samples were centrifuged twice (160 g for 5 min and then 25,000 g for 15 min) at 4°C. Equivalent proteins were loaded to 8% (BK_{Ca} α-subunit) or 12% (BK_{Ca} β1-subunit) SDS-PAGE for size separation. A 1:200 dilution of the rabbit polyclonal antibody against BK_{Ca} α- or BK_{Ca} β1-subunit (Alomone Labs, Jerusalem, Israel) was used to

incubate with the nitrocellulose membranes, respectively. The membrane was then incubated for 45 min with infrared (IR)-labeled secondary antibodies (LI-COR) in PBS-T containing 0.01% SDS. The Odyssey infrared imaging system (LI-COR) was used to detect the bound antibody, and Scion image (Scion, Frederick, MD, USA) was used to analyze the densitometry of bands.

Evaluation of BK_{Ca} mRNA expression by real-time PCR

Total RNA was extracted from cerebral arteries according to our previous report (Sun *et al.* 2015). Total RNA was extracted from cerebral arteries with TRIzol reagent (Invitrogen) according to manufacturer's protocol. The concentration and purity of total RNA were determined by measuring absorbance at 260 and 280 nm using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Incorporated). Real-time quantitative PCR analysis was performed, and the endogenous β -actin was used to evaluate the efficiency. The primer pairs of BK_{Ca} α -subunit (KCNMA1) were Forward-5'-CAA GAT GGA TGC GCT CAT CA-3' and Reverse-5'-TAG AAA TTC TGG CAG GAT TC-3'. The primer pairs of BK_{Ca} β 1-subunit (KCNMB1) were Forward-5'-AAG CTG GTG ATG GCC CAG AA-3' and Reverse-5'-TGG TTT TGA TCC CGA GTG TC-3'. The primer pairs of β -actin were Forward-5'-TCA

GGT CAT CAC TAT CGG CAAT-3' and Reverse-5'-AAA GAA AGG GTG TAA AAC GCA-3'.

Statistical analysis

Except the data of body weight that are given as means \pm S.D., all other data are expressed as means \pm S.E.M. One-way ANOVA was used to determine the differences of BK_{Ca} whole-cell current densities followed by a SNK *Post Hoc*. Student's *t*-test was used to determine significant differences of body weights, blood glucose, *Am* and *Po* in different groups. A value of $P < 0.05$ was considered to be statistically significant.

Results

Physical characteristics of experimental animals

As previously reported (Wu *et al.* 2012, Moghaddam *et al.* 2013), the diabetic rat model has been established successfully for blood glucose significantly increased and body weights markedly decreased in diabetic rats at 8 weeks after STZ injection, respectively. Chronic administration of 50 mg/kg/day berberine for 8 weeks did not affect the blood glucose and body weight in both CON and Diabetic rats, respectively. Chronic administration of

Table 1 Body weight and fasting blood glucose in CON, CON+berberine, Diabetic and Diabetic+berberine rats at 8 weeks after STZ injection in Experiment I, Experiment II and Experiment III.

	Initial		8 week after STZ injection	
	Body weight (g)	Blood glucose (mM)	Body weight (g)	Blood glucose (mM)
Experiment I				
CON ($n=10$)	189.0 \pm 21.8	5.2 \pm 1.2	498.0 \pm 58.2	4.8 \pm 3.3
CON + 50 mg/kg/day berberine ($n=10$)	188.5 \pm 15.3	4.8 \pm 2.3	501.5 \pm 65.1	4.9 \pm 2.1
Diabetic ($n=10$)	192.0 \pm 16.6	5.1 \pm 1.9	273.0 \pm 82.8*	18.6 \pm 5.9*
Diabetic + 50 mg/kg/day berberine ($n=10$)	185.0 \pm 15.5	4.8 \pm 2.5	278.0 \pm 68.9*	18.2 \pm 6.3*
Experiment II				
CON ($n=20$)	185.0 \pm 22.8	3.9 \pm 1.2	523.0 \pm 65.2	4.8 \pm 1.2
CON + 100 mg/kg/day berberine ($n=20$)	186.5 \pm 20.3	4.1 \pm 1.5	502.5 \pm 72.1	3.7 \pm 1.1
Diabetic ($n=20$)	180.0 \pm 25.6	4.6 \pm 1.4	263.0 \pm 62.7*	21.9 \pm 3.8*
Diabetic + 100 mg/kg/day berberine ($n=20$)	177.0 \pm 19.5	4.1 \pm 1.1	321.0 \pm 68.7#.*	15.2 \pm 4.3#.*
Experiment III				
CON ($n=10$)	190.0 \pm 21.0	4.5 \pm 1.2	523.0 \pm 47.9	5.8 \pm 1.6
CON + 200 mg/kg/day berberine ($n=10$)	186.9 \pm 13.6	4.8 \pm 1.5	418.5 \pm 67.2*	3.3 \pm 1.6*
Diabetic ($n=10$)	194.0 \pm 27.7	5.4 \pm 1.8	296.0 \pm 72.2*	17.8 \pm 6.5*
Diabetic + 200 mg/kg/day berberine ($n=10$)	198.0 \pm 21.8	4.9 \pm 1.6	398.0 \pm 65.8#.*	11.5 \pm 3.3#.*

Berberine was administrated with different dosages of 50, 100 and 200 mg/kg/day for 8 weeks in Experiment I, Experiment II and Experiment III, respectively. * $P < 0.05$ vs CON and # $P < 0.05$ vs Diabetic rats were determined by Student's *t*-test in different groups.

CON, control rats; CON+berberine, control rats administrated with berberine; Diabetic, diabetic rats; Diabetic+berberine, diabetic rats administrated with berberine.

100 mg/kg/day berberine for 8 weeks markedly decreased the blood glucose and increased the body weight of diabetic rats, respectively, which is well in accordance with previous reports (Wu *et al.* 2012, Moghaddam *et al.* 2013, Ma *et al.* 2016). Chronic administration of 200 mg/kg/day berberine for 8 weeks significantly decreased blood glucose in Diabetic rats and CON rats (Table 1). These results indicated that dosage of 100 mg/kg/day berberine is appropriate and has an effective hypoglycemic activity in diabetic rats.

Chronic administration of 100 mg/kg/day berberine for 8 weeks significantly reduced systolic and diastolic blood pressure in diabetic rats

As compared with control rats, diabetic rats showed a significant increase in systolic and DBP (Fig. 1), which is consistent with previous report (Fajloun *et al.* 2015). Chronic administration of 50 mg/kg/day berberine did

not affect the blood pressure in both CON and Diabetic rats, respectively. Chronic administration of 100 mg/kg/day berberine significantly reduced the blood pressure in Diabetic rats, whereas did not affect the blood pressure in CON rats. Chronic administration of 200 mg/kg/day berberine significantly decreased blood pressure not only in Diabetic rats but also in CON rats. These results indicated that dosage of 100 mg/kg/day berberine is effective for reducing blood pressure in diabetic rats.

Chronic administration of 100 mg/kg/day berberine for 8 weeks significantly induced the relaxation of middle cerebral artery in diabetic rats

As compared with that in CON, endothelium-dependent relaxation to acetylcholine (Ach, Figs 2A and 3A) and endothelium-independent relaxation to sodium nitroprusside (SNP, Figs 2B and 3B) of middle

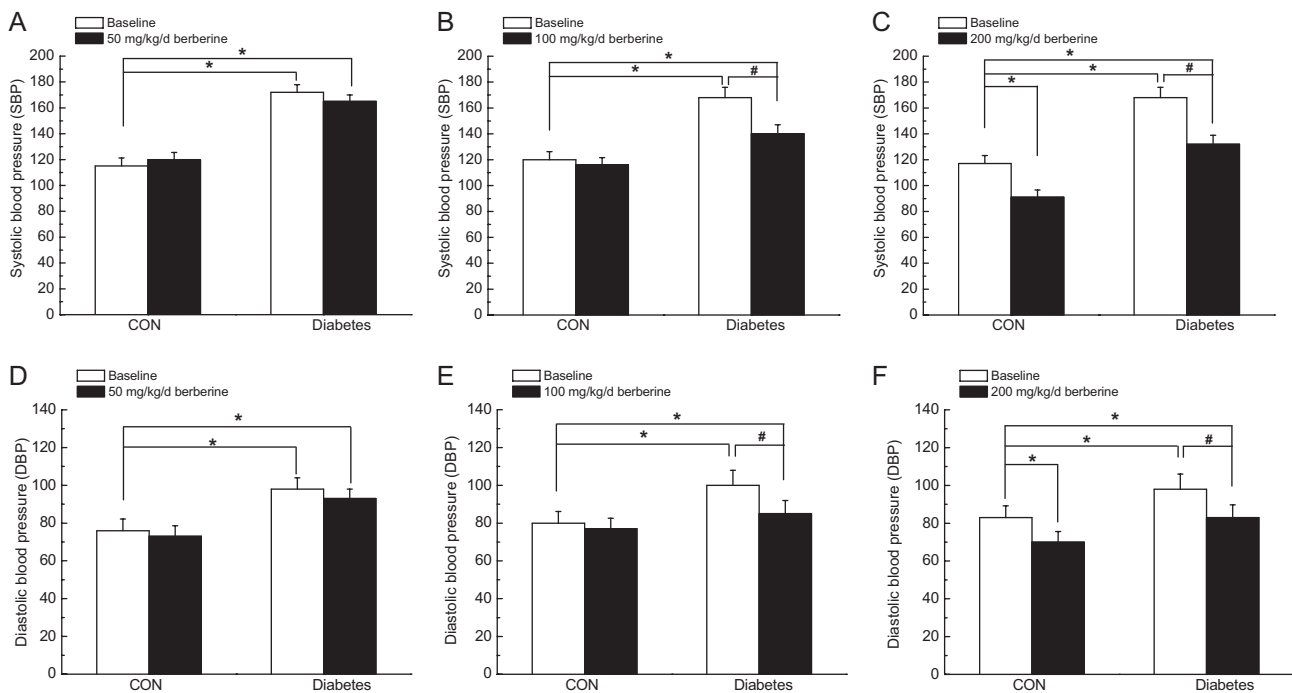


Figure 1

Comparison of systolic and diastolic blood pressure from CON, CON+berberine, Diabetic and Diabetic+berberine rats. Berberine was administrated with different dosages of 50, 100 and 200 mg/kg/day for 8 weeks in Experiment I, Experiment II and Experiment III, respectively. Chronic administration of 50 mg/kg/day berberine had no obvious effects on systolic and diastolic blood pressure in CON or Diabetic rats, respectively. However, chronic administration of 100 mg/kg/day berberine significantly reduced the systolic and diastolic blood pressure in Diabetic rats, whereas did not affect the blood pressure in CON rats. In addition, chronic administration of 200 mg/kg/day berberine significantly decreased systolic and diastolic blood pressure in both CON and Diabetic rats, respectively. CON, control rats; CON+berberine, control rats administrated with berberine; Diabetic, diabetic rats; and Diabetic+berberine, diabetic rats administrated with berberine. Values are expressed as means \pm S.E.M. and $n=10$ animals in each group. * $P<0.05$ vs CON rats and # $P<0.05$ vs Diabetic rats were determined by Student's *t*-test in different groups.

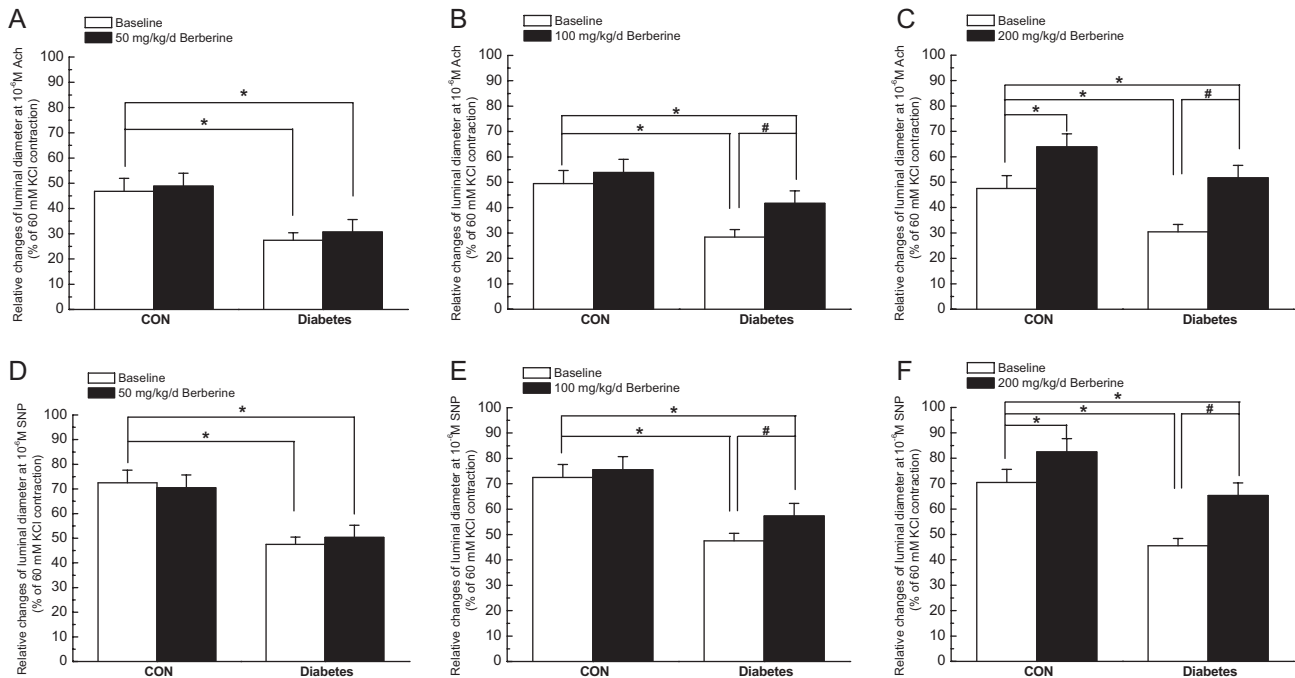


Figure 2

Comparison of relaxation in response to 10^{-6} M acetylcholine (Ach, A) and 10^{-6} M sodium nitroprusside (SNP, B) in middle cerebral artery isolated from CON, CON+berberine, Diabetic and Diabetic+berberine rats, respectively. Berberine was administrated with different dosages of 50, 100 and 200 mg/kg/day for 8 weeks in Experiment I, Experiment II and Experiment III, respectively. Chronic administration of 50 mg/kg/day berberine had no obvious effects on relaxation to Ach (A) and SNP (B) in CON or Diabetic rats, respectively. However, chronic administration of 100 mg/kg/day berberine significantly increased the relaxation to Ach (A) and SNP in Diabetic rats, whereas did not change the relaxation in CON rats. In addition, chronic administration of 200 mg/kg/day berberine significantly enhanced the relaxation to Ach (A) and SNP (B) in both CON and Diabetic rats, respectively. CON, control rats; CON+berberine, control rats administrated with berberine; Diabetic, diabetic rats; and Diabetic+berberine, diabetic rats administrated with berberine. Values are expressed as means \pm s.e.m. and $n=8$ animals in each group. * $P < 0.05$ vs CON rats and # $P < 0.05$ vs Diabetic rats were determined by Student's *t*-test in different groups.

cerebral artery both significantly decreased in diabetic rats, which are in accordance with previous reports (Vallejo *et al.* 2014, Wang *et al.* 2009a,b, 2015). In Experiment I, chronic treatment with 50 mg/kg/day berberine for 8 weeks did not obviously affect relaxation in both CON and Diabetic rats, respectively (Fig. 2). In Experiment II, chronic administration of 100 mg/kg/day berberine for 8 weeks significantly increased the relaxation of middle cerebral artery to Ach (Fig. 3A) and SNP (Fig. 3B) in diabetic rats, respectively, whereas did not affect the relaxation in CON rats. For example, 10^{-6} M Ach markedly increased the relative changes of luminal diameter from $(28.5 \pm 2.9)\%$ in diabetic rats to $(41.7 \pm 5.0)\%$ in Diabetic+berberine rats. 10^{-6} M SNP significantly enhanced the relative changes of luminal diameter from $(47.5 \pm 2.9)\%$ in Diabetic rats to $(57.3 \pm 4.9)\%$ in Diabetic+berberine rats (Fig. 2B). In Experiment III, chronic treatment with 200 mg/kg/day berberine for 8 weeks significantly increased vascular relaxation not only in Diabetic rats but also in CON

rats (Fig. 2). These results indicated that dosage of 100 mg/kg/day berberine is appropriate for increasing relaxation in Diabetic rats. Therefore, we selected 100 mg/kg/day of berberine as the working dosage in the animal study.

Chronic administration of 100 mg/kg/day berberine for 8 weeks markedly increased the BK_{Ca} whole-cell current densities and BK_{Ca} open probability of cerebral VSMCs in diabetic rats

As shown in Fig. 4A, whole-cell BK_{Ca} currents of VSMCs in Diabetic rats significantly decreased as compared with those in CON (Fig. 4A), which is consistent with the previous reports (Dong *et al.* 2008, Yi *et al.* 2014). Chronic administration of berberine (100 mg/kg/day) for 8 weeks significantly increased BK_{Ca} whole-cell current densities of VSMCs in Diabetic rats, whereas did not change the BK_{Ca} current densities of VSMCs in CON rats (Fig. 4B). As shown in Fig. 5A, BK_{Ca} open probability

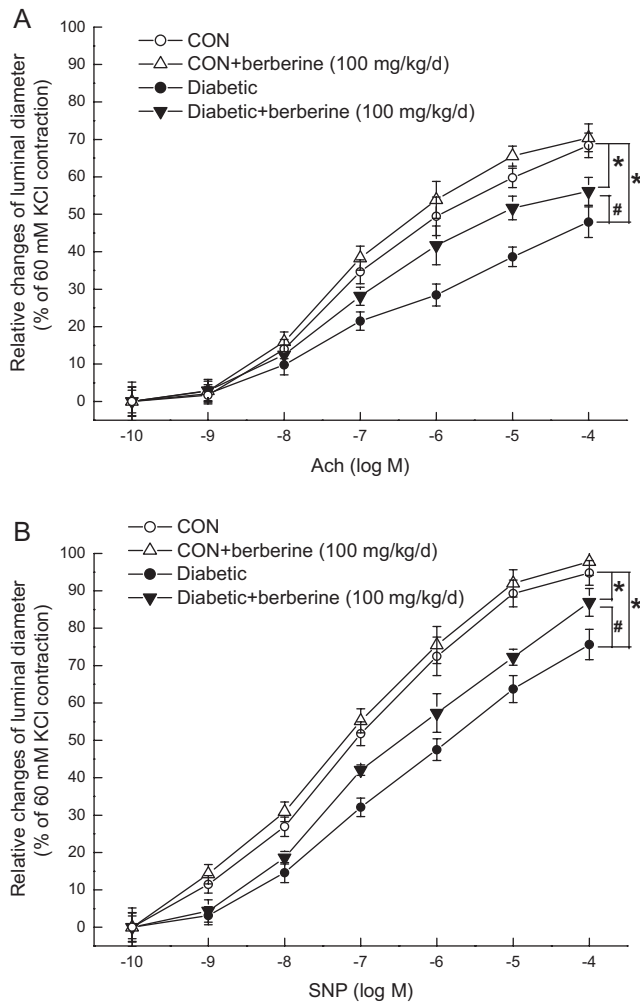


Figure 3 Chronic administration of 100 mg/kg/day berberine significantly increased the endothelium-dependent relaxation to acetylcholine (Ach, A) and the endothelium-independent relaxation to sodium nitroprusside (SNP, B) in middle cerebral artery isolated from CON, CON+berberine, Diabetic and Diabetic+berberine rats. Relaxation of middle cerebral artery were represented as the percentage of luminal diameter relative to the constriction diameter induced by 60 mM KCl. CON, control rats; CON+berberine, control rats administrated with 100 mg/kg/day berberine; Diabetic, diabetic rats; and Diabetic+berberine, diabetic rats administrated with 100 mg/kg/day berberine. Values are expressed as means \pm s.e.m. and $n=8$ animals in each group. * $P<0.05$ vs CON rats and # $P<0.05$ vs Diabetic rats were determined by one-way ANOVA in different groups.

of VSMCs in Diabetic rats significantly reduced, which is consistent with the previous reports (Dong *et al.* 2008, Yi *et al.* 2014). Chronic administration of 100 mg/kg/day berberine for 8 weeks significantly increased BK_{Ca} open probability of VSMCs in Diabetic rats. In contrast, there were no significant differences in BK_{Ca} channel unitary amplitude among different groups.

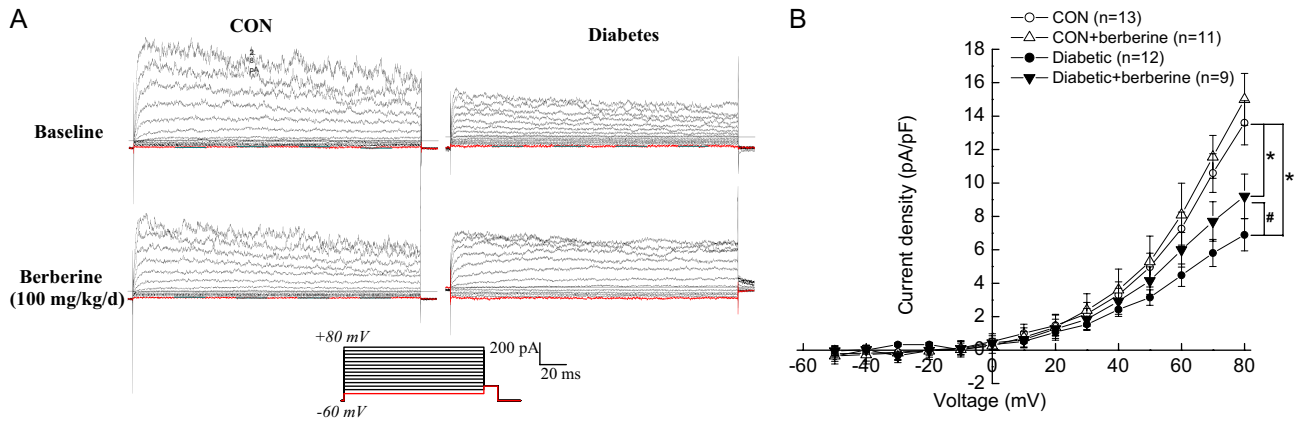
These results suggested that 100 mg/kg/day berberine treatment significantly increased the BK_{Ca} whole-cell and single-channel activities of VSMCs isolated from Diabetic rats.

Chronic administration of 100 mg/kg/day berberine for 8 weeks significantly increased the expression of BK_{Ca} β 1-subunit at protein and mRNA levels in cerebral arteries isolated from diabetic rats

As shown in Figs 6 and 7, there were no significant differences in α -subunit expression of BK_{Ca} at protein (Fig. 6B) and mRNA levels (Fig. 7A) among different groups. In contrast, β 1-subunit protein (Fig. 6B) and mRNA (Fig. 7B) expressions of BK_{Ca} channel significantly decreased in cerebral arteries of diabetic rats, which are consistent with the previous reports (Dong *et al.* 2008, Yi *et al.* 2014). Chronic administration of 100 mg/kg/day berberine for 8 weeks significantly increased the reduced β 1-subunit expression of BK_{Ca} channel in Diabetic rats. When the control rats were treated with 100 mg/kg/day berberine, there were no significant differences in the BK_{Ca} β 1-subunit expression between CON+berberine and CON rats. These results suggested that berberine treatment significantly increased the expressions of BK_{Ca} β 1-subunit at protein and mRNA levels in cerebral arteries isolated from Diabetic rats.

Acute application of berberine directly induced the relaxation of cerebral arteries isolated from normal rats by direct activation of BK_{Ca} channel under hyperglycemia condition

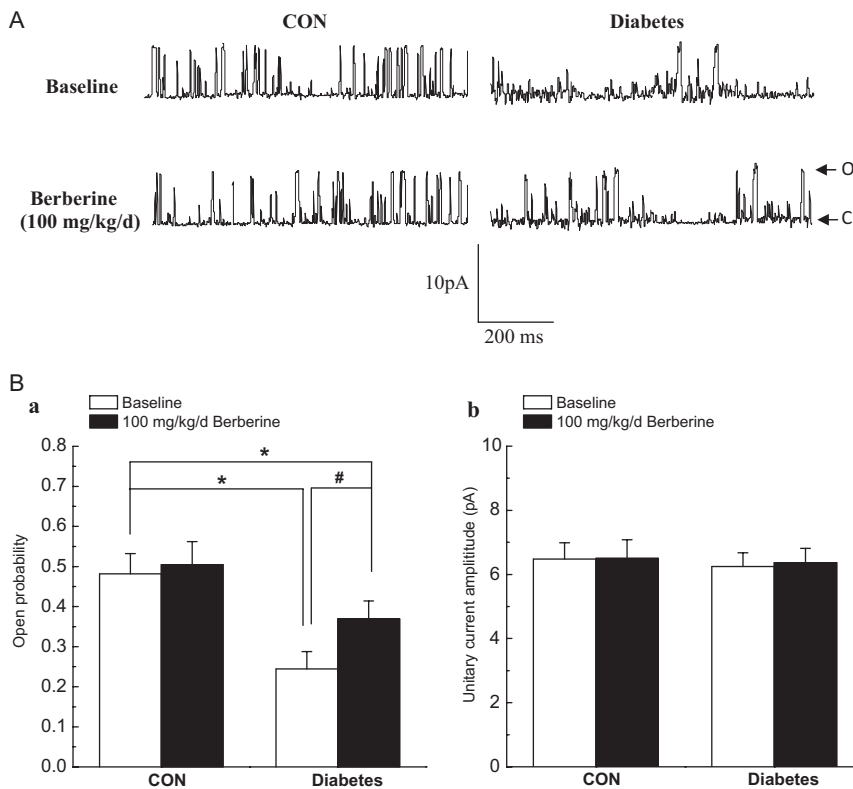
The middle cerebral artery was isolated from normal control rats. Then the relaxation was investigated in response to the acute effects of berberine under hyperglycemia condition. As shown in Fig. 8A, extracellular application of high glucose (20 mM D-glucose for 2–7 min) (Ma *et al.* 2016) obviously induced the contraction. When the contraction was stable, acute extracellular application of 10 μ M berberine markedly induced the relaxation in the presence of 20 mM D-glucose. The dose of acute extracellular application of 10 μ M berberine was used in this study according to our pre-experiments and our previous reports (Ma *et al.* 2016). Subsequently, extracellular application of 100 nM IBTX, the specific blocker of BK_{Ca} channel, significantly blocked the berberine-induced

**Figure 4**

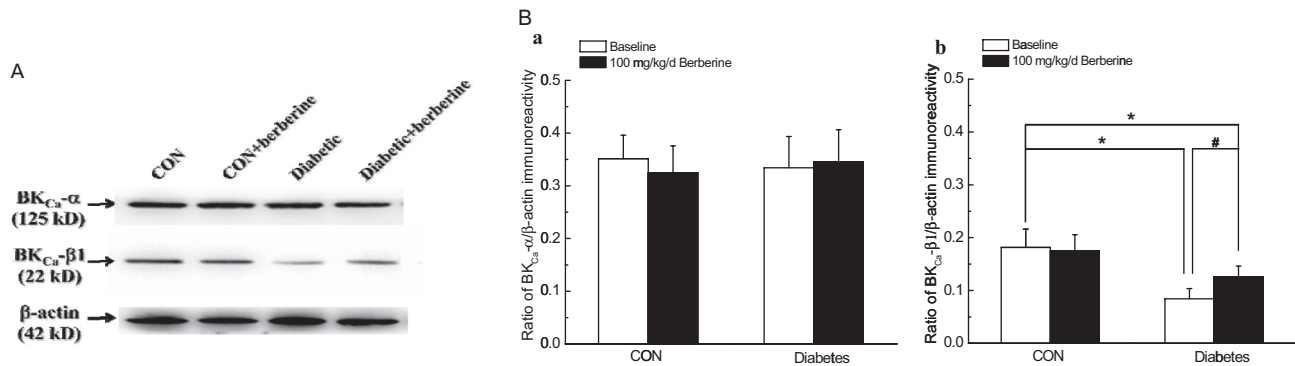
Chronic administration of 100 mg/kg/day berberine markedly increased whole-cell BK_{Ca} current densities of cerebral VSMCs isolated from Diabetic rats. Representative recording traces were used to show the characteristics of whole-cell BK_{Ca} currents (A), and the mean $I-V$ curves were further expressed in terms of current densities (B) in CON, CON+berberine, Diabetic and Diabetic+berberine rats. CON, control rats; CON+berberine, control rats administrated with 100 mg/kg/day berberine; Diabetic, diabetic rats; and Diabetic+berberine, diabetic rats administrated with 100 mg/kg/day berberine. Values are means \pm s.e.m. with the number of cells recorded in parentheses. * $P < 0.05$ vs CON rats and # $P < 0.05$ vs Diabetic rats were determined by one-way ANOVA in different groups. A full color version of this figure is available at <http://dx.doi.org/10.1530/JME-17-0014>.

relaxation of middle cerebral artery. In addition, fresh cerebrovascular VSMCs were isolated from normal control rats. As shown in Fig. 9, extracellular application of the 20 mM D-glucose significantly inhibited BK_{Ca} whole-cell currents in VSMCs. Subsequently, acute extracellular application of 10 μ M berberine markedly

increased the BK_{Ca} whole-cell currents in the presence of 20 mM D-glucose. These findings suggested that hyperglycemia induce constriction and then the acute extracellular application of berberine could directly induce relaxation by activation of BK_{Ca} channel under hyperglycemia condition.

**Figure 5**

Chronic administration of 100 mg/kg/day berberine significantly increased the BK_{Ca} open probability of cerebral VSMCs in Diabetic rats. Representative recording traces were used to show the BK_{Ca} single-channel currents at +40 mV test potential from cell-attached patches in different groups (A). The mean open probability (P_o) and the unitary amplitude (A_m) were further expressed in different groups (B). CON, control rats; CON+berberine, control rats administrated with 100 mg/kg/day berberine; Diabetic, diabetic rats; and Diabetic+berberine, diabetic rats administrated with 100 mg/kg/day berberine. Values are expressed as means \pm s.e.m. and at least $n = 10$ cells recorded in each group. * $P < 0.05$ vs CON rats and # $P < 0.05$ vs Diabetic rats were determined by Student's t -test in different groups (O, open state, C close state).

**Figure 6**

Chronic administration of 100 mg/kg/day berberine markedly increased the BK_{Ca} β1-subunit protein expressions of BK_{Ca} channel in cerebral arteries isolated from Diabetic rats. Representative band was used to show the protein expressions of BK_{Ca} α- and BK_{Ca} β1-subunit in different groups (A). Normalized band intensities are shown as a percentage of the β-actin density (B). CON, control rats; CON + berberine, control rats administrated with 100 mg/kg/day berberine; Diabetic, diabetic rats; and Diabetic + berberine, diabetic rats administrated with 100 mg/kg/day berberine. Values are expressed as means ± s.e.m. from 4 independent experiments, and each sample is based on tissue pooled from 3 to 4 animals. **P* < 0.05 vs CON rats and #*P* < 0.05 vs Diabetic rats were determined by Student's *t*-test in different groups.

Discussion

There are two principal and novel findings in the present work. First, chronic administration of 100 mg/kg/day berberine not only lowered blood glucose but also reduced blood pressure and improved vasodilation in STZ-induced diabetic rats, which suggested that berberine treatment may provide a combinational therapy for lowering blood glucose and reducing blood pressure in diabetes at the same time. Secondly, berberine treatment markedly increased the function and expression of BK_{Ca} channel β1-subunit in cerebral VSMCs isolated from STZ-induced diabetic rats or when exposed to hyperglycemia condition, which may be the underlying mechanism responsible for the vascular protection of berberine in diabetes.

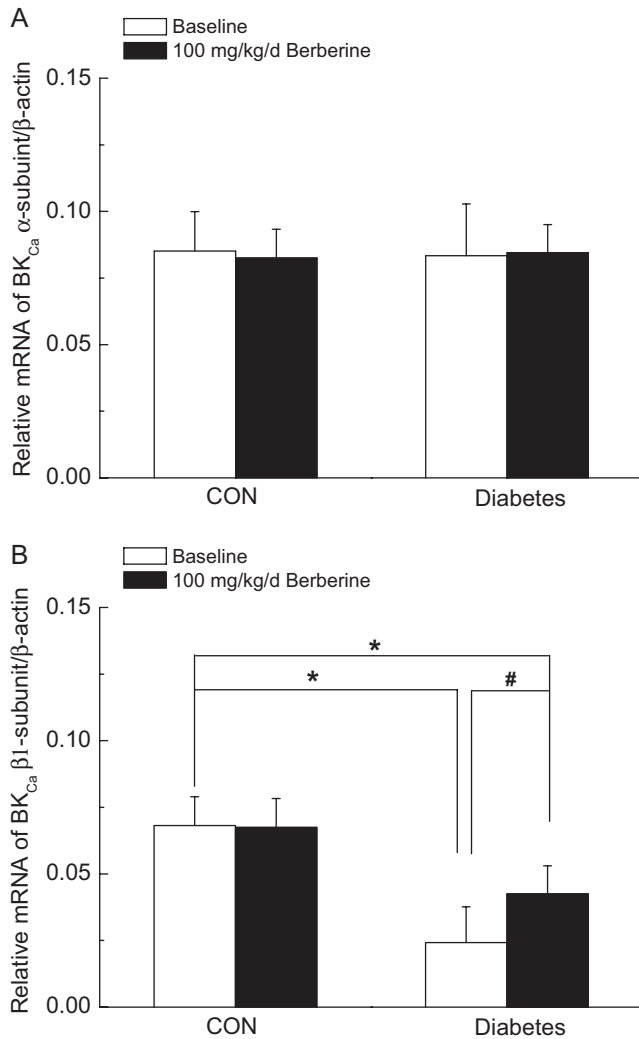
Hyperglycemia, hypertension and impaired BK_{Ca} channel in diabetic vascular complication

STZ is an antibiotic, is able to destroy pancreatic beta cells and is widely used experimentally in rodents to induce a type 1 or 2 diabetic phenotype. The main characteristic of type 1 diabetes is an autoimmune destruction of the pancreatic beta cells, leading to lack of insulin production. Therefore, a single high dose of STZ (doses range from 100 to 200 mg/kg in mice or 35–65 mg/kg in rats) is commonly used to induce a rapid ablation of beta cells and then the hyperglycemia (King 2012). Type 2 diabetes is characterized by insulin resistance and the inability of beta cells to sufficiently compensate. In addition, human obesity is closely linked to type 2 diabetes development. Therefore, many animal models of type 2 diabetes are

established by high-fat diet plus low dose of STZ (doses range from 20 to 40 mg/kg per day in mice or rats) to induce insulinitis and the reduction in insulin secretion capacity (Zhang *et al.* 2008).

Vascular disease is one of the important factors for the increased risks of stroke, heart attack and organ damage in diabetic patients (Fernández-Velasco *et al.* 2014). Hyperglycemia and hypertension that are considered to be the two hallmark features in diabetes for most diabetic patients with elevated blood pressure will require two or more agents to induce hypotensive and hypoglycemic effects (Mancia 2005, Tillin *et al.* 2011). It is not clear why the diabetic subjects have an increased susceptibility to hypertension, but the mechanisms may involve the impaired vascular function in vasodilation and vasoconstriction (Mancia 2005).

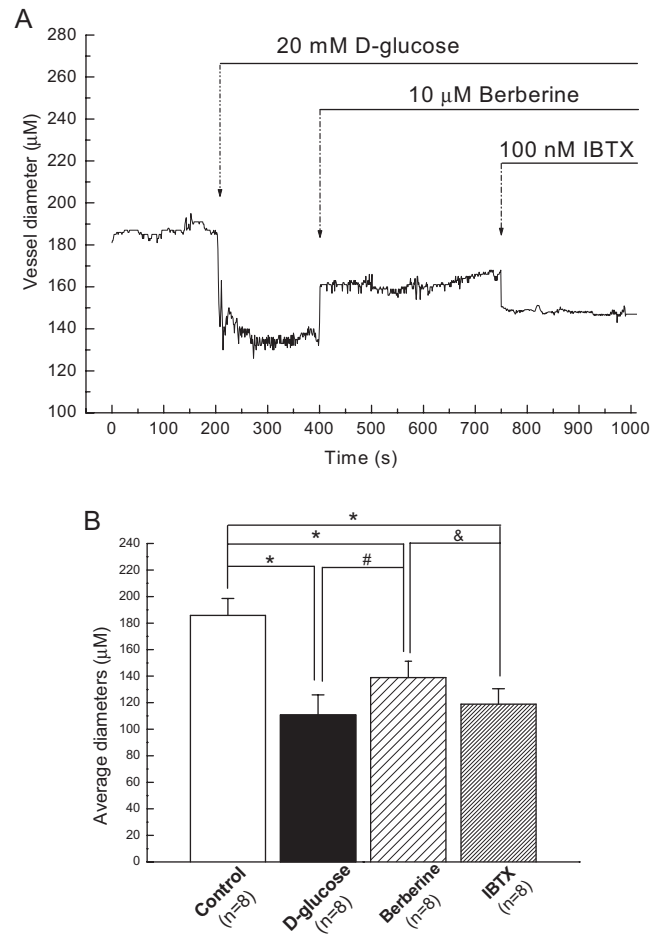
It has been demonstrated that hyperglycemia directly impaired vasodilation by impairing BK_{Ca} channel in smooth muscle, and then led to the hypertension in diabetes (Tousoulis *et al.* 2013, Fernández-Velasco *et al.* 2014, Gutterman & Durand 2014). BK_{Ca} channel is an important protective mechanism to regulate the dynamic equilibrium between vascular constriction and relaxation (Lazuko *et al.* 2014). Activation of BK_{Ca} channel induces K⁺ efflux and promotes VSMC relaxation by limiting Ca²⁺ influx (Gutterman & Durand 2014, Nystoriak *et al.* 2014, Yi *et al.* 2014). Vascular BK_{Ca} channel is composed of pore-forming α-subunit and accessory β1-subunit. The presence of BK_{Ca}-β1 is known to regulate the channel kinetics by increasing Ca²⁺ and voltage sensitivity. In particular, it is found that impaired BK_{Ca} channel is associated with

**Figure 7**

Chronic administration of 100 mg/kg/day berberine markedly increased the BK_{Ca} β1-subunit mRNA expressions of BK_{Ca} channel in cerebral arteries isolated from Diabetic rats. Relative mRNA of α- and β1-subunit are shown as a percentage of the β-actin mRNA in (A) and (B), respectively. CON, control rats; CON+berberine, control rats administrated with 100 mg/kg/day berberine; Diabetic, diabetic rats; and Diabetic+berberine, diabetic rats administrated with 100 mg/kg/day berberine. Values are expressed as means ± s.e.m. from 6 independent experiments. **P* < 0.05 vs CON rats and #*P* < 0.05 vs Diabetic rats were determined by Student's *t*-test in different groups.

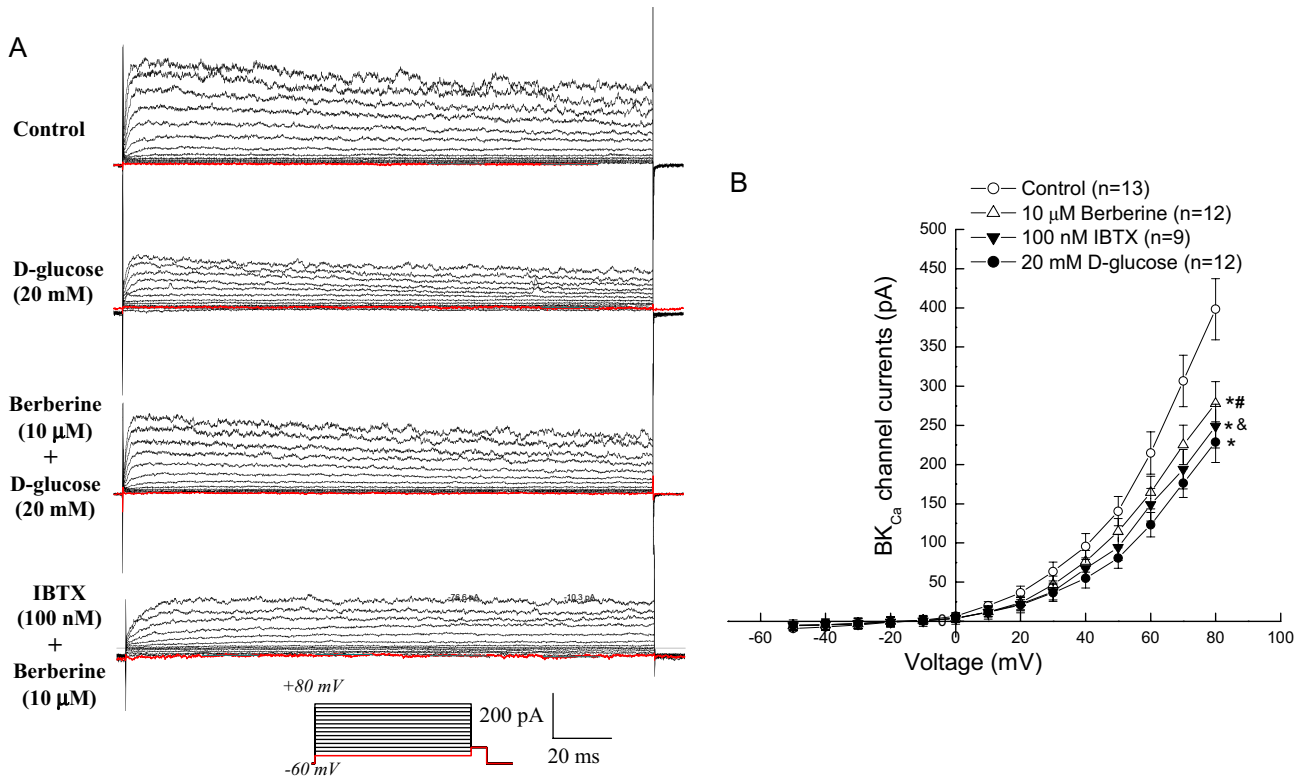
a significant downregulation of BK_{Ca} β1-subunit, but not BK_{Ca} α-subunit, which resulted in the uncoupling of Ca²⁺ sparks and spontaneous transient outward current (Dong *et al.* 2008, Mori *et al.* 2011).

As compared with that in CON, we observed that the rat model of STZ-induced diabetic rats showed a higher blood glucose level (Table 1) and an elevated blood pressure (Fig. 1) with a significant decrease in the

**Figure 8**

Acute application of berberine directly induced vasodilation of middle cerebral artery isolated from CON rats by activation of BK_{Ca} channel under hyperglycemia condition. Representative diameters of middle cerebral artery were recorded before and after application of 20 mM D-glucose, 10 μM berberine and 100 nM iberiotoxin (IBTX, the specific antagonist of BK_{Ca} channel), respectively (A). Summary data for average diameters before and after application of D-glucose, berberine and IBTX (B). 20 mM D-glucose induced the contraction, and then 10 μM berberine was added as indicated dose when the contraction was stable. The berberine-induced relaxation was diminished by the addition of IBTX, the specific blocker of BK_{Ca} channel. Values are means ± s.e.m. and *n* = 8 from 6 animals. **P* < 0.05 vs control condition, #*P* < 0.05 vs high glucose condition and &*P* < 0.05 vs Berberine were determined by Student's *t*-test in different groups.

relaxation (Figs 2 and 3). In addition, BK_{Ca} whole-cell currents (Fig. 4), single-channel activities (Fig. 5) and the expressions of BK_{Ca} β1-subunit at protein (Fig. 6) and mRNA levels (Fig. 7) significantly decreased in cerebral VSMCs isolated from STZ-induced diabetic rats. Correspondingly, we also observed that acute application of hyperglycemia directly induced the constriction (Fig. 8) and decreased the function of BK_{Ca} channel

**Figure 9**

Acute application of berberine directly activated BK_{Ca} currents in VSMCs isolated from normal control rats. Representative traces of BK_{Ca} channel current in VSMCs when exposed to 20 mM D-glucose and then subsequent acute application of 10 μ M berberine in the presence of 20 mM D-glucose (A). Summarized data indicated the BK_{Ca} whole-cell currents before and after application of 20 mM D-glucose and then 10 μ M berberine in the presence of 20 mM D-glucose (B). Values are means \pm S.E.M. with the number of cells recorded in parentheses. * $P < 0.05$ vs control condition, # $P < 0.05$ vs high glucose condition and & $P < 0.05$ vs Berberine were determined by Student's *t*-test in different groups. A full color version of this figure is available at <http://dx.doi.org/10.1530/JME-17-0014>.

(Fig. 9) in cerebral arteries isolated from normal control rats. Our results are in agreement with previous report that hyperglycemia impaired BK_{Ca} β 1-subunit, which led to the diabetic vascular dysfunction (Dong *et al.* 2008, Gutterman & Durand 2014, Nystoriak *et al.* 2014, Yi *et al.* 2014).

The berberine treatment reduced blood pressure and improved dilation in diabetic rats by direct activation of BK_{Ca} channel in VSMCs

After oral administration, berberine can be absorbed through the gastrointestinal tract, where the gut microbiota converts berberine into its absorbable form of dihydroberberine. Dihydroberberine is then reverted immediately to berberine through oxidization. It has been demonstrated that berberine is predominantly distributed in the liver and metabolized by cytochrome P450. The blood level of orally administered berberine in

rats plateaued at 4–24 h, and the maximal levels in the liver were achieved at 12 h. Finally, berberine excreted by the hepatobiliary system and kidneys in the form of metabolites (Feng *et al.* 2015). Berberine has been used in the treatment of diabetes for its reducing glucose uptake, regulating gluconeogenesis, modulating glucose handling and increasing insulin secretion and insulin sensitivity (Ni *et al.* 2015, Pang *et al.* 2015). In addition, berberine was found to exert a cardiovascular protection in diabetes for alleviating endothelial injury by reducing NADPH oxidase activity (Wu *et al.* 2012) and decreasing the formation of advanced glycation end products (AGEs) (Hao *et al.* 2011). Furthermore, it has been demonstrated that berberine also improved the endothelium-dependent vasodilation by stimulating the adenosine monophosphate-activated protein kinase (AMPK) pathway (Wang *et al.* 2009a,b) and increasing the phosphorylation of endothelial nitric oxide synthase (eNOS) (Wang *et al.* 2009a,b, Vallejo *et al.* 2014). In the present work, chronic administration of

100 mg/kg/day berberine not only significantly reduced glucose levels (Table 1), but also reduced blood pressure (Fig. 1) and enhanced the endothelium-dependent or endothelium-independent relaxation in diabetic rats (Figs 2 and 3). In addition, chronic administration of 100 mg/kg/day berberine markedly increased the BK_{Ca} whole-cell current densities (Fig. 4), enhanced the single-channel activity (Fig. 5), and increased the BK_{Ca} β1-subunit expressions at protein (Fig. 6) and mRNA levels (Fig. 7) in VSMCs isolated from diabetic rats. Correspondingly, acute application of 10 μM berberine could directly induce relaxation (Fig. 8) and activated BK_{Ca} currents (Fig. 9) under hyperglycemia in VSMCs isolated from normal control rats under hyperglycemia condition. Ach relaxed the pre-contracted artery ring in concentration-dependent manner, which was abolished by L-NAME, a NOS inhibitor and by removal of the endothelium, indicating that the relaxation was endothelium dependent and mediated by NO production (Diederich *et al.* 1990). In addition, SNP is most widely used as nitric oxide donors. NO has been documented to directly activate BK_{Ca} channel, which makes a major contribution to smooth muscle relaxation. NO also may increase intracellular levels of cyclic GMP and activate cGMP-dependent protein kinase (PKG). PKG may activate native or cloned BK_{Ca} channels through phosphorylation of channels (Ma *et al.* 2010). Therefore, high glucose impaired the vascular NO production and relaxation in diabetes, and our results clearly suggested berberine treatment reduced blood pressure and improved the relaxation in STZ-induced diabetic rats by direct activation of BK_{Ca} channel in VSMCs.

Practical implications of the present study

Several studies and new guidelines indicated that 2 or more antihypertensive agents could reduce blood pressure and show a concomitant reduction of cardiovascular risk in diabetic subjects with hypertension (Tillin *et al.* 2011, Ettehad *et al.* 2016). Here, we observed for the first time that berberine, a traditional herb, could provide a combinational therapy for lowering blood glucose and reducing blood pressure in diabetic rats at the same time. However, we also observed that berberine treatment did not restore the blood pressure (Fig. 1), blood glucose (Table 1), body weight (Table 1), cerebrovascular relaxation (Figs 2 and 3), and function and expression of BK_{Ca} channels (Figs 4, 5 and 6) in diabetic rats to the normal control level. Our results are in agreement with the present research that berberine treatment could be

a good alternative or at least supplement to classical hypoglycemic and hypotensive agents to diabetes (Li *et al.* 2014, Ni *et al.* 2015). However, berberine treatment does not take place on the basis of oral hypoglycemic agent completely, such as metformin, although berberine has been demonstrated to have anti-diabetes effects (Rios *et al.* 2015, Liu *et al.* 2016). We supposed the mechanisms are very complicated, including the different administration doses of berberine, the animal model and studies in clinical trials. In addition, it has been reported that berberine could alleviate diabetic vascular dysfunction by acting on endothelial cells (Wang *et al.* 2009a,b, Hao *et al.* 2011, Ding *et al.* 2015). Our previous work demonstrated that berberine could alleviate the constriction of smooth muscle by reducing the intracellular Ca²⁺ in diabetic rats (Ma *et al.* 2016). In complement of previous studies, the present study provided evidence that berberine could also induce the relaxation of smooth muscle by activation of BK_{Ca} channel in diabetic rats. It is notable that we observed that only the dosage of 100 mg/kg/day berberine is effective for reducing blood glucose and blood pressure in diabetic rats, whereas did not change blood glucose and blood pressure in CON rats. Therefore, the dosage of berberine is very important for the treatment in diabetes.

Study limitations

In the present study, we demonstrated that long-term exposure to hyperglycemia impaired the relaxation of middle cerebral artery (Figs 2 and 3) by the decreasing the function (Figs 4 and 5) and expression of BK_{Ca} β1-subunit at protein and mRNA levels (Figs 6 and 7) in Diabetic rats. In addition, we also observed that short-term exposure to hyperglycemia for 2–7 min obviously constricted the middle cerebral artery (Fig. 8) and decreased the function of BK_{Ca} channel (Fig. 9). However, we did not investigate the expression of BK_{Ca} β1-subunit at protein and mRNA levels when exposed to short-term hyperglycemia in the present study. It has been reported that short-term exposure to hyperglycemia for 2 h impaired the bradykinin-induced relaxation in human subcutaneous arteries and for 3 h attenuated the phenylephrine-induced contraction in rat aortic rings (Vorn & Yoo 2017). Therefore, it is reasonable to assume that short-term exposure to hyperglycemia for only 2–7 min did not change the expression of BK_{Ca} β1-subunit. We still wish to investigate whether short-term exposure to hyperglycemia could change the protein expression of BK_{Ca} channel in future work.

Conclusion

The present study provided initial evidences for the first time that berberine contributes to reducing blood pressure and improving vasodilation in STZ-induced diabetic rats by activation of BK_{Ca} channel in smooth muscle cells, which might provide a novel therapeutic approach to treat vascular complications in diabetic patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Yu-Guang Ma, Liang Liang and Man-Jiang Xie conceived and designed the experiments. Yu-Guang Ma, Liang Liang, Yin-Bin Zhang, Yun-Gang Bai and Zhi-Jun Dai performed the experiments. Liang Liang and Zhong-Wei Wang analyzed the data. Man-Jiang Xie and Zhong-Wei Wang wrote the paper.

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