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The vasodilatory effect of the antidiabetic drug linagliptin via inhibition of Rho-associated protein kinase in aortic smooth muscle

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ABSTRACT

Aims: The vasodilatory effects of the anti-diabetic drug, linagliptin in phenylephrine-precontracted aortic rings were investigated.
Materials and methods: Male New Zealand White rabbits were used in the experiment and its arterial tone was measured by using myogragh system.
Key findings: Linagliptin induced vasodilation in a concentration-dependent manner. The vasodilatory effect of linagliptin was not affected by the absence of the endothelium, or by pretreatment with a nitric oxide synthase inhibitor (L-NAME) or a small-conductance Ca^{2+}-activated K^+ channel inhibitor (apamin). Moreover, application of the adenylyl cyclase inhibitor SQ22536, protein kinase A (PKA) inhibitor KT5720, guanylyl cyclase inhibitor ODQ, or protein kinase G (PKG) inhibitor KT5823 did not alter the vasodilatory effect of linagliptin. However, inhibition of Rho-associated protein kinase by Y-27632 significantly attenuated linagliptin-induced vasodilation. Ion channel involvement in the vasodilatory effect of linagliptin was also investigated. Pretreatment with the vascular K^+ channel inhibitors glibenclamide (ATP-sensitive K^+ channels), Ba^{2+} (inwardly rectifying K^+ channels), 4-AP (voltage-dependent K^+ channels), and paxilline (large conductance Ca^{2+}-activated K^+ channels) did not affect linagliptin-induced vasodilation. Furthermore, the *L*-type Ca^{2+} channel inhibitor, nifedipine, and the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump inhibitor, thapsigargin, did not change the vasodilatory effect of linagliptin. *Significance:* We suggests that linagliptin-induced vasodilation was mediated by the inhibition of Rho-associated kinase, but not with the endothelium, cAMP-PKA or cGMP-PKG-dependent signaling pathways, K^+ channels, Ca^{2+} influx, or SERCA pump.

1. Introduction

Diabetes mellitus (DM), characterized by increased blood glucose levels, is the most common metabolic syndrome. As a chronic disease, type 2 DM imposes a significant health burden on patients due to serious complications including nephropathy, retinopathy, ischemic heart disease, macrovascular, and microvascular diseases [1–3]. Among the serious complications, long-term vascular complications involving cardiovascular diseases are associated with high mortality in patients with type 2 DM [4]. Several new drugs and drug classes have become available after decades of drug development for type 2 DM [5]. The inhibition of dipeptidyl peptidase-4 (DPP-4) is among the most widely used strategies to treat type 2 DM. DPP-4 is a ubiquitous multifunctional enzyme that is expressed in several organs that play crucial roles in metabolism, nutrition, and immune responses [6]. Furthermore, DPP-4 rapidly cleaves incretins such as glucose-dependent insulinotropic polypeptide and GLP-1. Therefore, DPP-4 inhibitor agents enhance incretin levels, thereby regulating the physiological control of glucose levels [7]. The DPP-4 inhibitor linagliptin effectively controls glucose levels and improves endothelial function in vessels [8,9]. Although linagliptin has been shown to improve endothelial cell function and induce vasodilation [10–12], its detailed vasodilatory mechanisms

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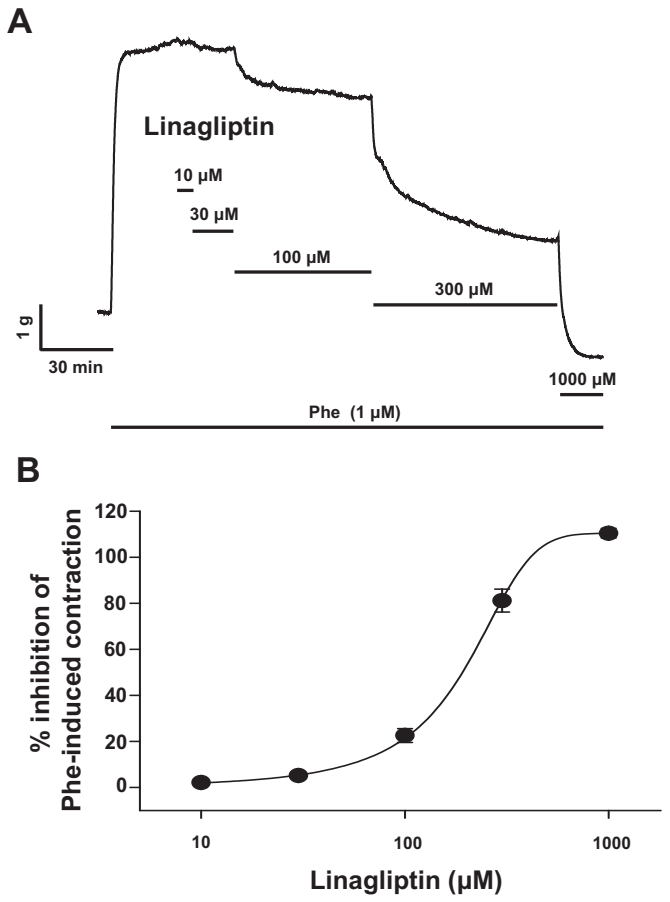

Fig. 1. The vasodilatory effect of linagliptin on Phe precontracted aortic rings. (A) The vasodilatory effect of various concentrations of linagliptin (10, 30, 100, 300, and 1000 μM) on aortic rings. (B) The concentration-dependent curve of linagliptin-induced vasodilation. $n = 8$. The n means the number of aortic rings isolated from different rabbits.

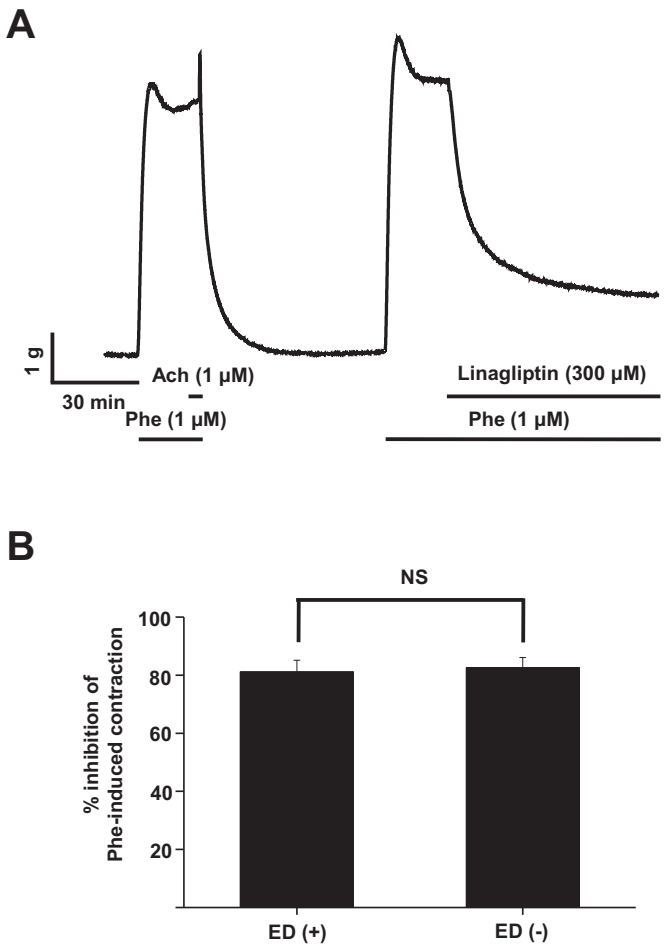

Fig. 2. The vasodilatory effect of linagliptin in endothelium-intact and -denuded aortic rings. (A) The vasodilatory effect of linagliptin on endothelium-denuded aortic rings. Complete absence of endothelial cells was confirmed by the application of acetylcholine, which induced further vasoconstriction. (B) Comparison of linagliptin-induced vasodilation in aortas with intact and denuded endothelium. $n = 5$. NS = not significant (endothelium-intact vs. endothelium-denuded aortic rings). The n means the number of aortic rings isolated from different rabbits.

have not been investigated.

The small GTP-binding protein, Rho A, regulates a wide range of fundamental cell functions such as gene expression, vesicle trafficking, cell division, adhesion, survival, and migration [13]. Rho-associated protein kinase (ROCK), a serine/threonine kinase involved in smooth muscle contraction, is an important downstream effector of Rho A. For this reason, the Rho A/ROCK signaling pathway is a major regulator of vascular tone [14,15]. Furthermore, alterations in ROCK-related signaling pathways or functions are closely associated with the onset of vascular diseases including hypertension [16–18]. Therefore, given the physiological relevance of ROCK in vascular function and the anti-diabetic effect of linagliptin, the effects of linagliptin on ROCK and ROCK-related signaling pathways warrant further investigation.

We investigated the vasodilatory effect of linagliptin on the rabbit thoracic aorta. We found that linagliptin induced vasodilation in a concentration-dependent manner. Our findings show that the vasodilatory effect of linagliptin was mediated by the inhibition of ROCK, but not associated with the endothelium, cAMP/protein kinase A (PKA)- and cGMP/protein kinase G (PKG)-dependent signaling pathways, K^+ or Ca^{2+} channels, or SERCA pump.

2. Materials and methods

2.1. Blood pressure measurements

Systolic and diastolic blood pressures were measured using a non-invasive blood pressure monitoring system (Bionics Co., Ltd., South Korea) with a 30-mm wide cuff. The cuff was wrapped around the brachial artery and stabilized for 10 min before measurements were taken. Blood pressure was measured 2 h after linagliptin (0.1 mg/Kg) was injected into the rabbit's ear vein to allow the drug to reach the maximum plasma concentration.

2.2. Vessel preparation and measurement

All experimental procedures and protocols were conducted according to the guidelines of the Committee for Animal Experiments at Kangwon National University. Male New Zealand White rabbits (2.0 to 2.5 kg) were anesthetized by injection of heparin (70 U/kg) and sodium pentobarbitone (30 mg/kg) via the ear vein. The heart was quickly removed from the anesthetized rabbits and immersed in normal Tyrode's solution. The connective and perivascular adipose tissues were removed from the aorta and the vessel was cut to a length of ~1 cm under the stereomicroscope. The aortic rings were fixed between two wire hooks attached to a transducer and suspended in an organ bath chamber. The chamber contained physiological salt solution (PSS) bubbled with 95% O_2 and 5% CO_2 for at least 1 h and maintained at 37 °C. The aorta rings were stretched to 1 g tension using passive force after they were attached to the wire hooks. Prior to the experiments, the rings were

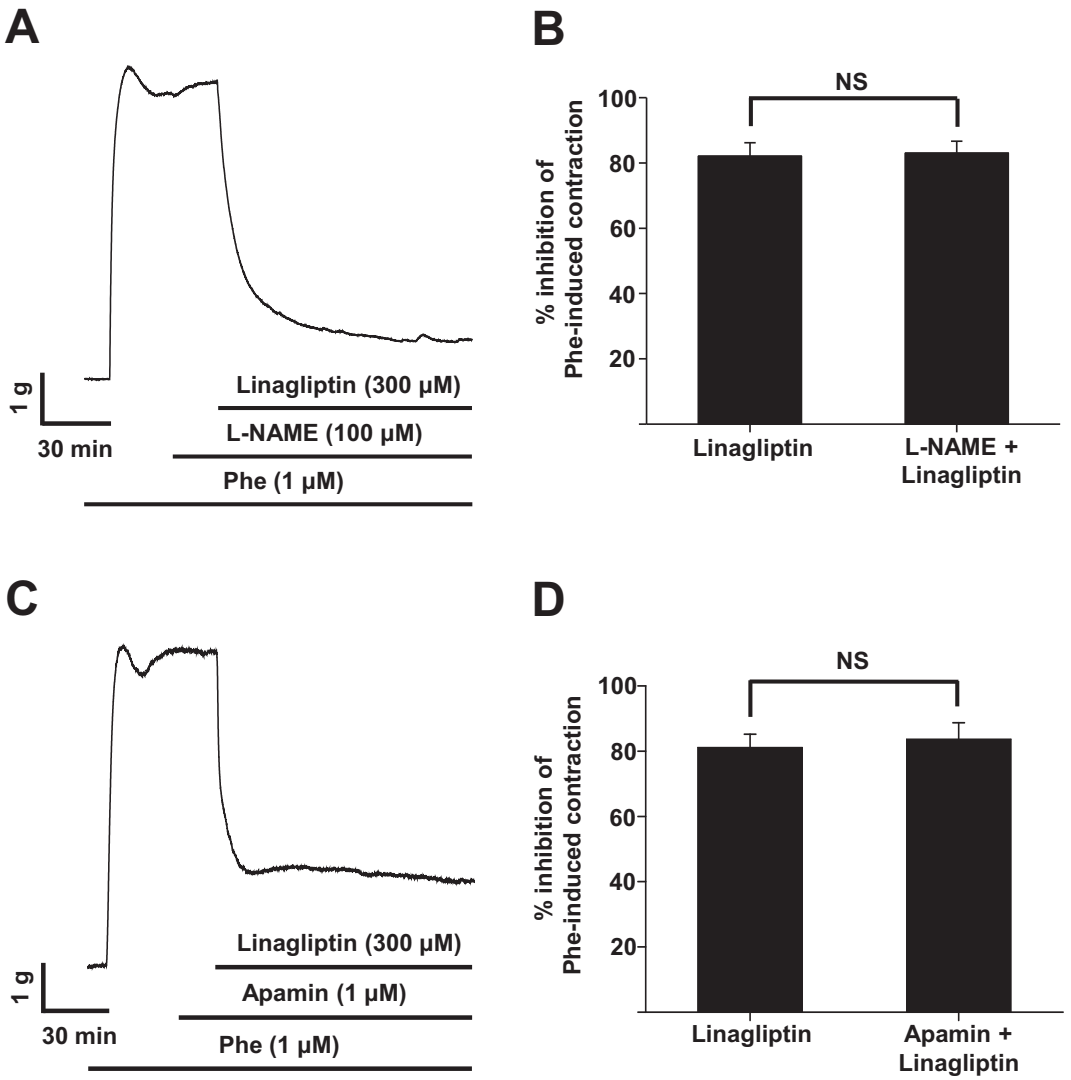

Fig. 3. Effects of the nitric oxide synthase inhibitor L-NAME and the SK_{Ca} channel blocker apamin on linagliptin-induced vasodilation in endothelium-intact aortic rings. (A) The vasodilatory effect of linagliptin in the presence of L-NAME. (B) Summary of the effects of L-NAME on linagliptin-induced vasodilation. $n = 6$. NS = not significant (linagliptin vs. L-NAME + linagliptin). The n means the number of aortic rings isolated from different rabbits. (C) The effect of apamin pretreatment on linagliptin-induced vasodilation. (D) Summary of the effects of apamin on linagliptin-induced vasodilation. $n = 5$. NS = not significant (linagliptin vs. apamin + linagliptin). The n means the number of aortic rings isolated from different rabbits.

exposed to a high K^+-PSS solution (80 mM) to test vessel viability. We also confirmed that phenylephrine (Phe)-induced contraction was stably maintained during the experiments (Supplementary Fig. 1). Endothelium-denuded arteries were obtained by passing air bubbles through the lumen of the intact arteries for 10 min to remove the endothelial cells.

2.3. Solutions and chemicals

The normal Tyrode's solution containing (mM): KCl 5.2, NaCl 141, $CaCl_2$ 1.7, NaH_2PO_4 0.36, HEPES 5.5, $MgCl_2$ 0.7, and Glucose 15.7, adjusted to pH 7.4 with NaOH. PSS containing (mM): KCl 5.2, NaCl 122, $CaCl_2$ 1.7, $NaHCO_3$ 23, KH_2PO_4 1.4, $MgSO_4$ 1.3, and Glucose 15.5 (pH 7.4). Linagliptin, Phe, 4-aminopyridine (4-AP), and $BaCl_2$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and dissolved in dimethyl sulfoxide (DMSO) or distilled water. Acetylcholine, paxilline, glibenclamide, SQ 22536, ODQ, KT 5720, KT 5823, L-NAME, apamin, nifedipine, thapsigargin, and Y-27632 were purchased from Tocris Cookson (Ellisville, MO) and dissolved in dimethyl sulfoxide (DMSO) or distilled water.

2.4. Statistical analyses

Statistical tests were conducted using Origin v.7.0 software (Microcal Software, Inc., Northampton, MA, USA). The data are expressed as means ± standard error of the mean. Unpaired Student's t-tests were used to evaluate statistical significance. P-values < 0.05 were considered to indicate statistical significance.

3. Results

3.1. Linagliptin induced vasodilation in a concentration-dependent manner

We found that linagliptin induced vasodilation in aortic rings pre-contracted with Phe in a concentration-dependent manner (Fig. 1A). Application of 100 and 300 μM linagliptin increased vasodilation by 22.60 and 81.17%, respectively (Fig. 1B). The vasodilatory effect of linagliptin was first observed at 10 μM, and concentrations higher than 1000 μM did not induce further vasodilation.

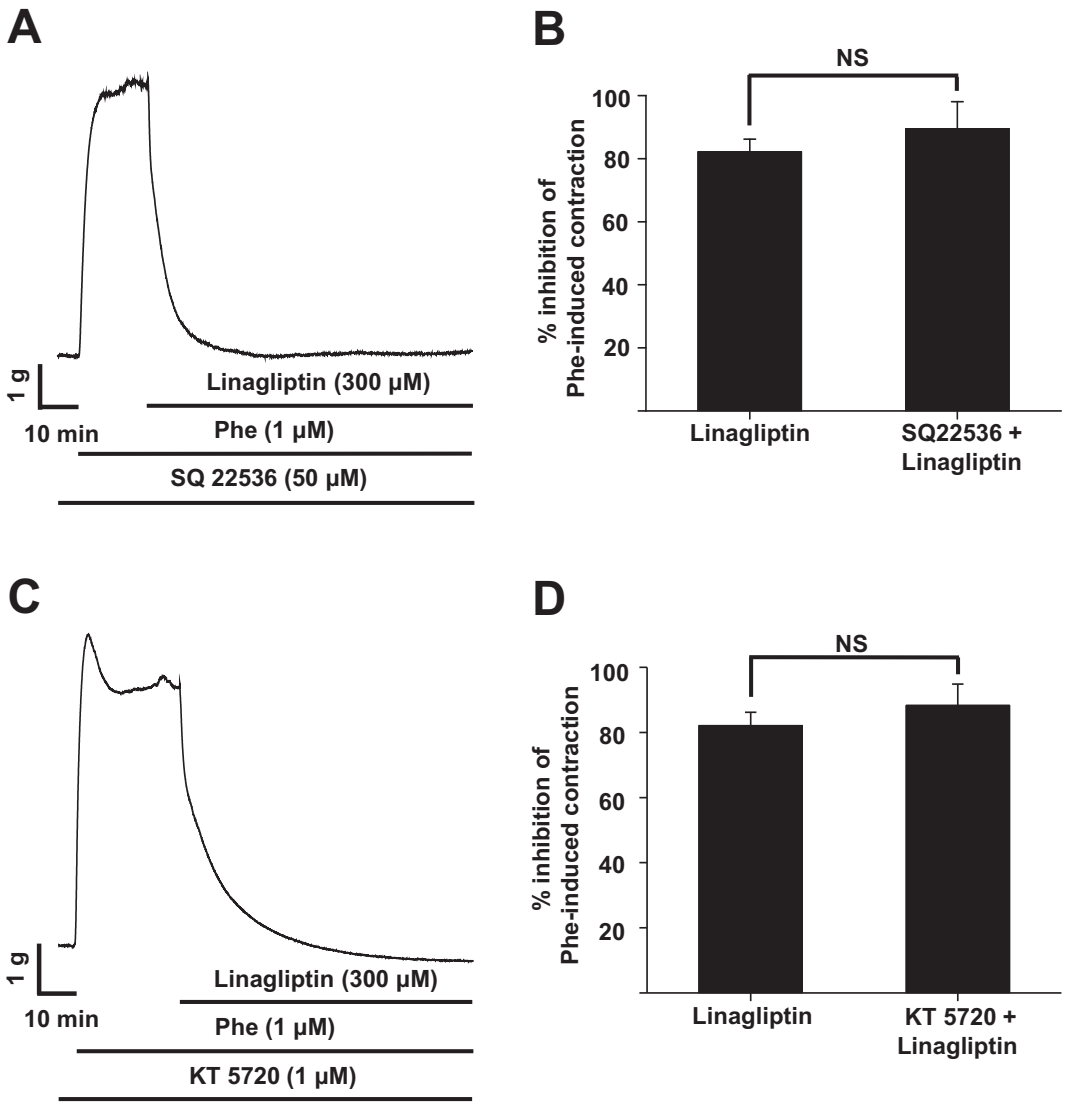

Fig. 4. Effects of the adenylyl cyclase inhibitor SQ22536 and the cAMP-dependent protein kinase inhibitor KT5720 on linagliptin-induced vasodilation. (A) Effect of SQ22536 pretreatment on linagliptin-induced vasodilation of Phe precontracted aortic rings. (B) Summary of the effects of SQ22536 on linagliptin-induced vasodilation. $n = 5$. NS = not significant (linagliptin vs. SQ22536 + linagliptin). The n means the number of aortic rings isolated from different rabbits. (C) Effect of KT5720 pretreatment on linagliptin-induced vasodilation of Phe precontracted aortic rings. (D) Summary of the effects of KT5720 on linagliptin-induced vasodilation. $n = 4$. NS = not significant (linagliptin vs. KT5720 + linagliptin). The n means the number of aortic rings isolated from different rabbits.

3.2. Endothelium involvement in linagliptin-induced vasodilation

We assessed linagliptin-induced vasodilation in endothelium-denuded aortic rings to determine the role of endothelium-dependent mechanisms. The absence of endothelial cells was confirmed by showing that the addition of acetylcholine further constricted the precontracted aortic rings. The absence of endothelial cells did not decrease the vasodilatory effect of linagliptin (Fig. 2A). In fact, the application of 300 μM linagliptin increased vasodilation by 81.17% in the endothelium-intact and by 82.61% in the endothelium-denuded arteries (Fig. 2B).

To further assess the role of the endothelium in linagliptin-induced vasodilation, we pretreated aortic rings with intact endothelium with the nitric oxide (NO) synthase inhibitor, L-NAME. Pretreatment with 100 μM L-NAME slightly increased vasoconstriction (Fig. 3A); however, the vasodilatory effect of linagliptin was not affected by L-NAME pretreatment (Fig. 3B). Moreover, pretreatment with 1 μM apamin, a small conductance Ca^{2+}-activated K^+ channel blocker, did not change linagliptin-induced vasodilation in the endothelium-intact aortic rings (Fig. 3C, D). These findings suggest that the vasodilatory effect of linagliptin is not associated with endothelium-dependent signaling pathways.

3.3. Involvement of cAMP/PKA- and cGMP/PKG-dependent signaling pathways in linagliptin-induced vasodilation

To investigate the involvement of cAMP/PKA-dependent signaling pathways in linagliptin-induced vasodilation, we inhibited adenylyl cyclase activity using the adenylyl cyclase inhibitor SQ22536. Pretreatment with 50 μM SQ22536 did not alter the vasodilatory effect of linagliptin (Fig. 4A, B). Similar to the SQ22536 findings, pretreatment with the PKA inhibitor KT5720 (1 μM) did not inhibit the vasodilatory effect of linagliptin (Fig. 4C, D).

To further assess the involvement of cGMP/PKG-dependent signaling pathways in linagliptin-induced vasodilation, we investigated the effects of pretreatment with the guanylyl cyclase inhibitor ODQ (10 μM) and the PKG inhibitor KT5823 (1 μM) on linagliptin-induced vasodilation. As shown in Fig. 5, pretreatment with ODQ (A, B) and KT5823 (C, D) did not alter the vasodilatory effect of linagliptin. Thus, our findings indicate that the cAMP/PKA- and cGMP/PKG-dependent

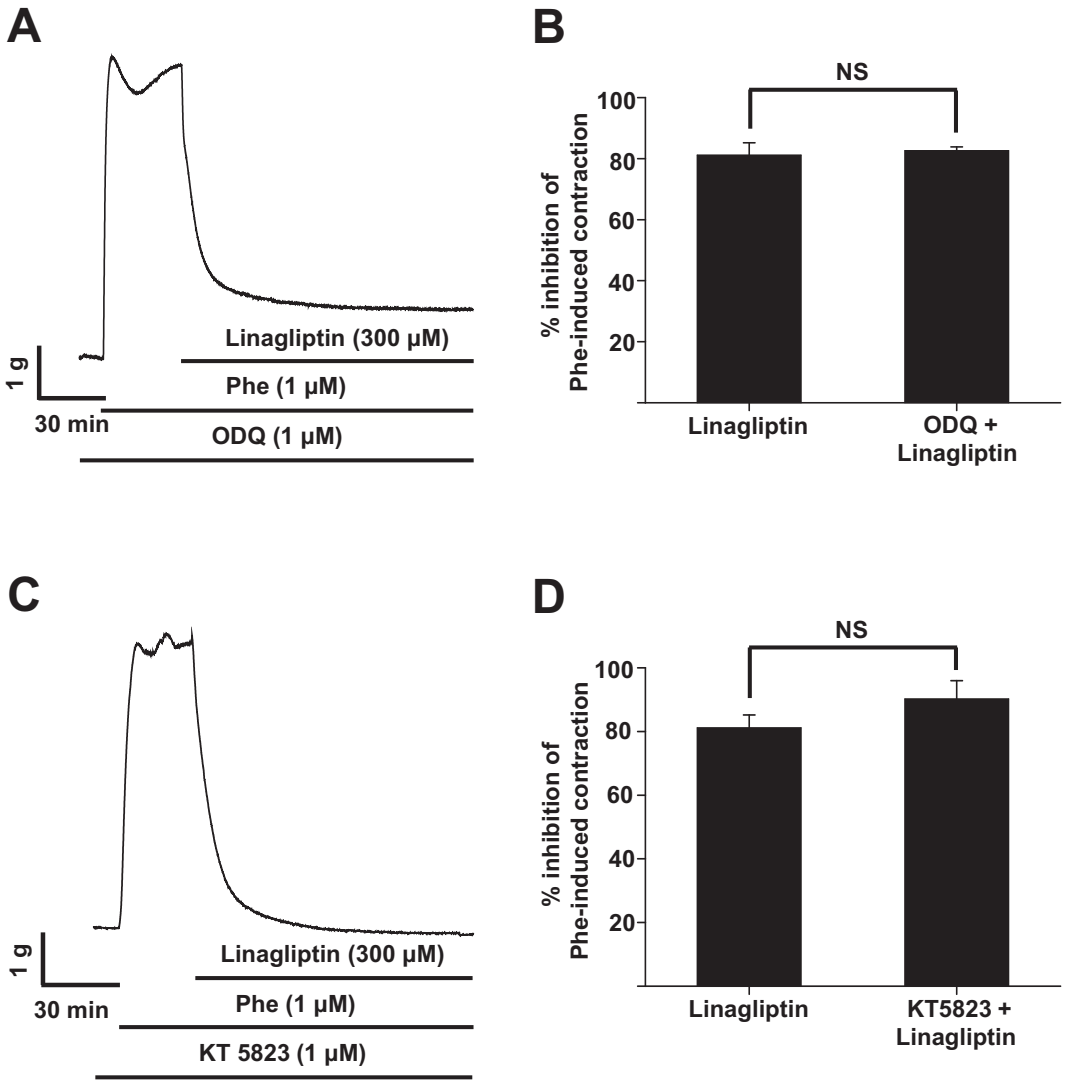

Fig. 5. Effects of the guanylyl cyclase inhibitor ODQ and the cAMP-dependent protein kinase inhibitor KT5823 on linagliptin-induced vasodilation. (A) The vasodilatory effect of linagliptin on precontracted aortic rings in the presence of ODQ. (B) Summary of the effects of ODQ on linagliptin-induced vasodilation. $n = 4$. NS = not significant (linagliptin vs. ODQ + linagliptin). The n means the number of aortic rings isolated from different rabbits. (C) The vasodilatory effect of linagliptin on precontracted aortic rings in the presence of KT5823. (D) Summary of the effects of KT5823 on linagliptin-induced vasodilation. $n = 6$. NS = not significant (linagliptin vs. KT5823 + linagliptin). The n means the number of aortic rings isolated from different rabbits.

signaling pathways do not contribute to the vasodilatory effect of linagliptin.

3.4. ROCK involvement in linagliptin-induced vasodilation

ROCK is a kinase belonging to the PKC/PKA/PKG family that is involved in the regulation of vascular contractility [19]. Therefore, we investigated the involvement of ROCK on linagliptin-induced vasodilation. Fig. 6A shows the vasodilatory effect of linagliptin in the presence of the ROCK inhibitor Y-27632. Pretreatment with 1 μM Y-27632 significantly reduced linagliptin-induced vasodilation from 81.17% to 44.09% (Fig. 6B), suggesting that the vasodilatory effect of linagliptin is closely associated with ROCK activity. To further investigate the involvement of ROCK in linagliptin-induced vasodilation, aortic rings were precontracted with U-46619, which induced sustained contraction through an increase in the amount of GTP-RhoA [20]. As shown in Fig. 6C, linagliptin induced vasodilation in aortic rings precontracted with U-46619. Linagliptin-induced vasodilation was significantly reduced by pretreatment with 1 μM Y-27632 (Fig. 6D and E). These results also suggest that the vasodilatory effect of linagliptin is closely associated with ROCK activity.

3.5. K^+ channel involvement in linagliptin-induced vasodilation

Activation of vascular K^+ channels causes vasodilation [21]. Therefore, we investigated whether any of the four types of K^+ channels expressed in vascular smooth muscle played a role in linagliptin-induced vasodilation. Pretreatment with the ATP-sensitive K^+ (K_{ATP}) channel inhibitor glibenclamide (10 μM) did not alter the vasodilatory effect of linagliptin (Fig. 7A, B). Application of the inwardly rectifying K^+ channel inhibitor Ba^{2+} (50 μM) further constricted the precontracted aortic rings (Fig. 7C); however, Ba^{2+} pretreatment did not affect the degree of linagliptin-induced vasodilation (Fig. 7D). Similarly, although the addition of the voltage-dependent K^+ channel inhibitor 4-aminopyridine (4-AP) further constricted the precontracted aortic rings (Fig. 7E), pretreatment did not attenuate the vasodilatory action of linagliptin (Fig. 7F). Furthermore, application of the large-conductance Ca^{2+}-activated K^+ channel inhibitor paxilline did not alter the vasodilatory effect of linagliptin (Fig. 7G, H). These findings suggest that the vasodilatory effect of linagliptin is independent of K^+

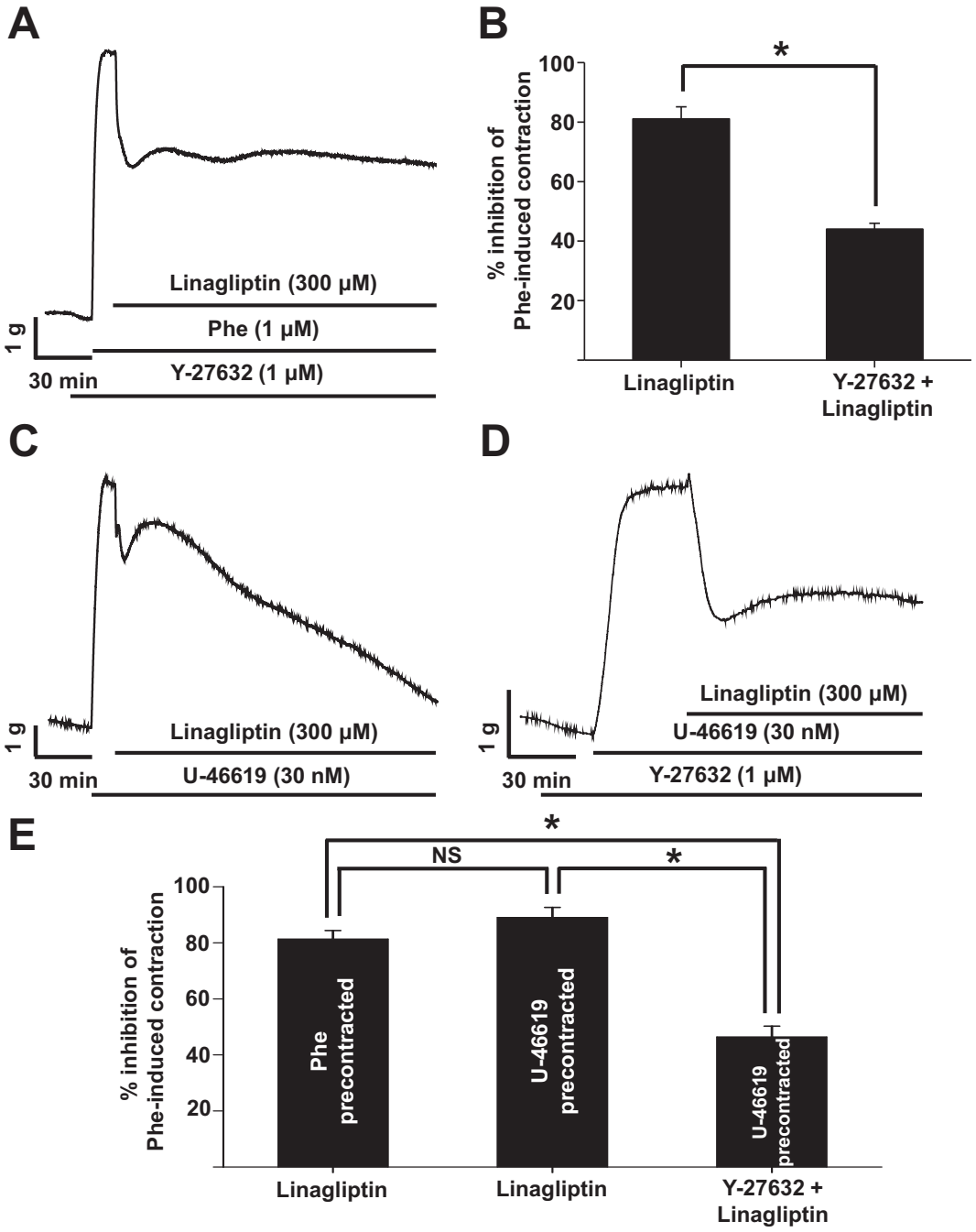

Fig. 6. Effects of the ROCK inhibitor Y-27632 on linagliptin-induced vasodilation. (A) The vasodilatory effect of linagliptin on precontracted aortic rings in the presence of Y-27632. (B) Summary of the effects of Y-27632 on linagliptin-induced vasodilation. $n = 6$. *$P < 0.05$ (linagliptin vs. Y-27632 + linagliptin). The n means the number of aortic rings isolated from different rabbits. (C) The vasodilatory effect of linagliptin on U-46619 precontracted aortic rings. (D) The vasodilatory effect of linagliptin on U-46619-precontracted aortic rings in the presence of Y-27632. (E) Summary of panel (C) and (D). $n = 4$. The n means the number of aortic rings isolated from different rabbits. NS = not significant (linagliptin, Phe-precontracted vs. linagliptin, U-46619-precontracted). *$P < 0.05$ (linagliptin, Phe-precontracted vs. Y-27632 + linagliptin, U-46619-precontracted; linagliptin, U-46619-precontracted vs. Y-27632 + linagliptin, U-46619-precontracted).

channel activation.

3.6. Involvement of Ca^{2+} channels and the SERCA pump in linagliptin-induced vasodilation

The precontracted aortic rings were pretreated with the *L*-type Ca^{2+} channel inhibitor nifedipine (10 μM) and the SERCA pump inhibitor thapsigargin (1 μM) to investigate the role of Ca^{2+} channels and SERCA pump in linagliptin-induced vasodilation. In fact, compared with high K^+-induced vasoconstriction, Phe-induced vasoconstriction was reduced by pretreatment with nifedipine or thapsigargin (Supplementary Fig. 2). However, the vasodilatory effect of linagliptin was not significantly altered by pretreatment with nifedipine (Fig. 8A, B) or thapsigargin (Fig. 8C, D), suggesting that linagliptin-induced vasodilation is not associated with Ca^{2+} channels or SERCA pump.

3.7. Linagliptin decreased blood pressure

To determine the effect of linagliptin-induced vasodilation on blood pressure, we measured blood pressure changes following the injection

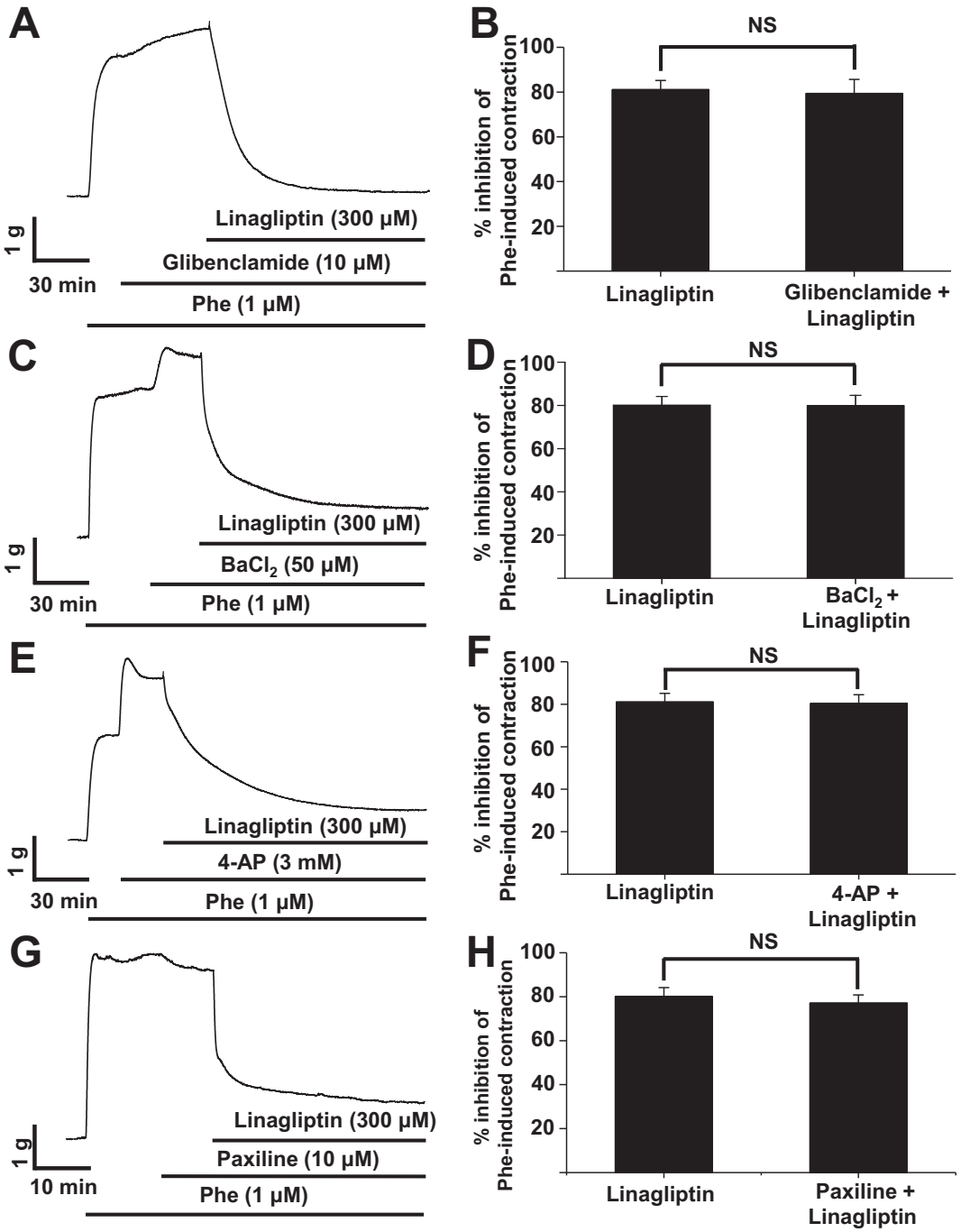

Fig. 7. Effects of K^+ channel (K_{ATP}, Kir, Kv, and BK_{Ca}) inhibition on linagliptin-induced vasodilation. (A) Involvement of the K_{ATP} channel in linagliptin-induced vasodilation. (B) Summary of the effects of glibenclamide on linagliptin-induced vasodilation. $n = 4$. NS = not significant (linagliptin vs. glibenclamide + linagliptin). (C) Involvement of the Kir channel in linagliptin-induced vasodilation. (D) Summary of the effects of Ba^{2+} on linagliptin-induced vasodilation. $n = 5$. NS = not significant (linagliptin vs. $BaCl_2$ + linagliptin). (E) Involvement of the Kv channel in linagliptin-induced vasodilation. (F) Summary of the effects of 4-AP on linagliptin-induced vasodilation. $n = 5$. NS = not significant (linagliptin vs. 4-AP + linagliptin). (G) Involvement of the BK_{Ca} channel in linagliptin-induced vasodilation. (H) Summary of the effects of paxilline on linagliptin-induced vasodilation. $n = 6$. NS = not significant (linagliptin vs. paxilline + linagliptin). All n means the number of aortic rings isolated from different rabbits.

of linagliptin (0.1 mg/kg) via the ear vein in rabbits. The administration of linagliptin reduced systolic and diastolic blood pressure from 130.26 ± 2.92 and 85.95 ± 2.14 mm Hg, respectively, at baseline to 104.54 ± 3.05 and 66.18 ± 2.58 mm Hg, respectively (Fig. 9).

4. Discussion

We found that linagliptin induced vasodilation in the rabbit thoracic aorta in a concentration-dependent manner. The vasodilatory effect of linagliptin was attenuated by the inhibition of ROCK, whereas the endothelium, cAMP/PKA and cGMP/PKG-dependent signaling pathways, K^+ and Ca^{2+} channels, and SERCA pump were not involved in linagliptin-induced vasodilation.

Type 2 DM is a metabolic disease in which blood glucose levels are not regulated. The rapid worldwide increase in type 2 DM has placed an enormous health and economic burden on society, and the associated

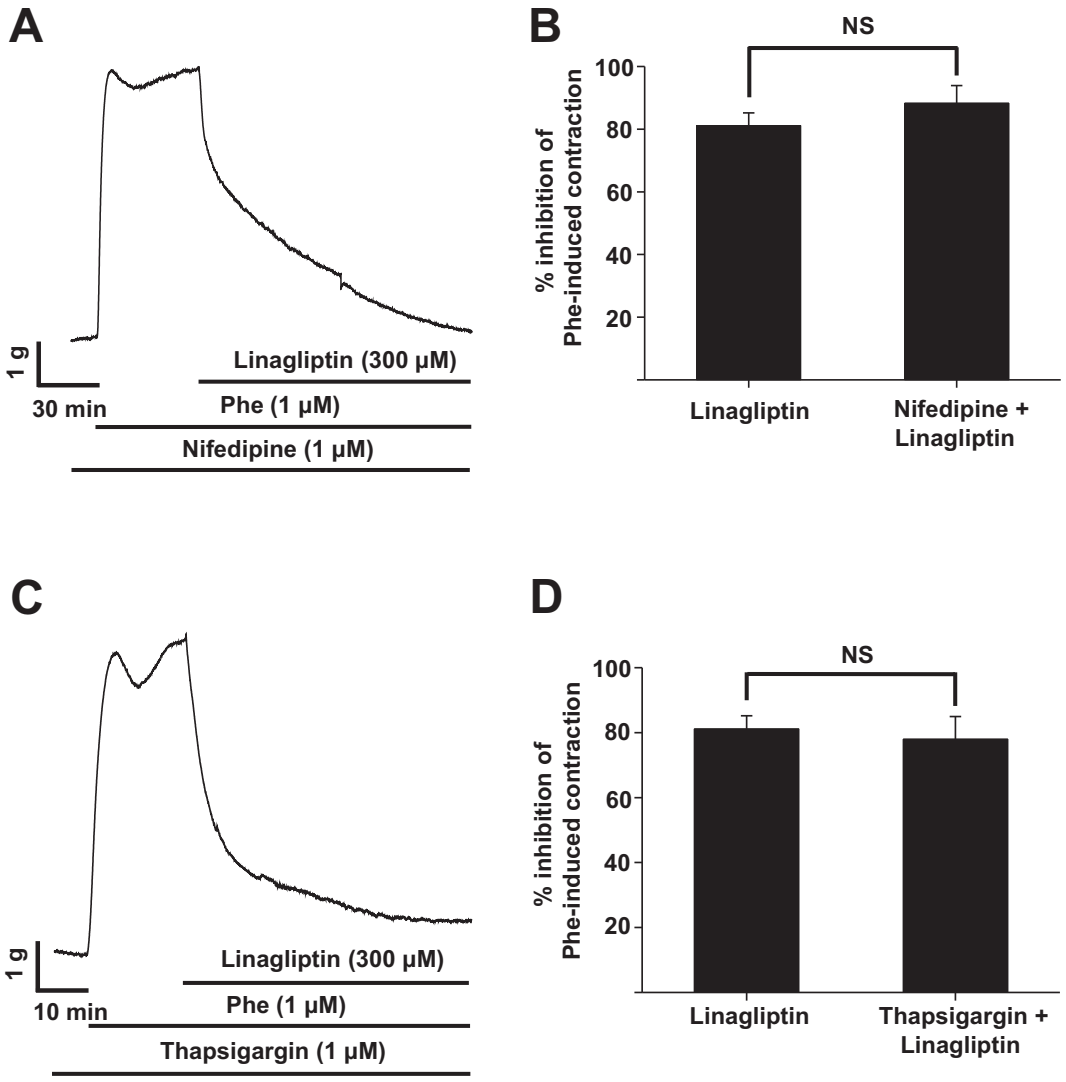

Fig. 8. Effects of the Ca^{2+} channel inhibitor nifedipine and the SERCA pump inhibitor thapsigargin on linagliptin-induced vasodilation. (A) The vasodilatory effect of linagliptin on precontracted aortic rings in the presence of nifedipine. (B) Summary of the effects of nifedipine on linagliptin-induced vasodilation. $n = 5$. NS = not significant (linagliptin vs. nifedipine + linagliptin). The n means the number of aortic rings isolated from different rabbits. (C) The vasodilatory effect of linagliptin on precontracted aortic rings in the presence of thapsigargin. (D) Summary of the effects of thapsigargin on linagliptin-induced vasodilation. $n = 4$. NS = not significant (linagliptin vs. thapsigargin + linagliptin). The n means the number of aortic rings isolated from different rabbits.

health complications are the cause of increased mortality. Therefore, effective control of blood glucose levels and prevention of serious health complications have been the driving forces behind the development of antidiabetic drugs. Several antidiabetic agents are currently available. Among these, the DPP-4 inhibitors are highly effective and widely used to treat DM. Besides the efficacy of DPP-4 inhibitors in treatment of DM, the effect of DPP-4 inhibitors on vascular tone have also been reported [22,23]. Specifically, linagliptin has been shown to increase eNOS availability, thus enhancing NO production, which may explain the vasodilation [12]. Linagliptin has also exhibited pleiotropic vasodilation through activation of the NO/cGMP signaling pathways independent of its glucose-lowering properties [10]. This study demonstrated that linagliptin induces vasodilation via the inhibition of ROCK without involvement of the NO-dependent signaling pathway, and it lowers blood pressure. We found that linagliptin induced vasodilation and lowered blood pressure suggesting that the drug may be effective for patients with DM who have hypertension. Conversely, prescribing linagliptin to patients with DM and hypotension may not be warranted.

Several signaling pathways are involved in the contraction-relaxation responses in vascular smooth muscle. Among these, ROCK signaling pathways are involved in vasocontractility. Vascular smooth muscle contraction is regulated by the phosphorylation and dephosphorylation of myosin light chain (MLC). ROCK inhibits MLC phosphatase, resulting in hypercontraction of vascular smooth muscle [24,25]. Therefore, our findings suggest that the vasodilatory effect of linagliptin is due to its inhibition of ROCK activity. In support of this hypothesis, pretreatment with the ROCK inhibitor Y-27532 significantly attenuated the vasodilatory effect of linagliptin, and Y-27632 applied alone before pre-constriction of the aortic rings induced vasodilation (Fig. 6). Moreover, ROCK activity may decrease the incidence of vascular diseases, including cardiovascular disease and arterial hypertension in particular [16,26]. Specifically, previous studies found increased ROCK activity in rats [17] and patients with hypertension [18]. Moreover, the inhibition of ROCK normalizes blood pressure in rat models of hypertension [27]. Similarly, we found that linagliptin reduced blood pressure. Therefore, the vasodilatory and blood pressure-lowering effects of linagliptin may be closely related to the inhibition of ROCK activity.

Arterial vasodilation is primarily mediated through endothelium-

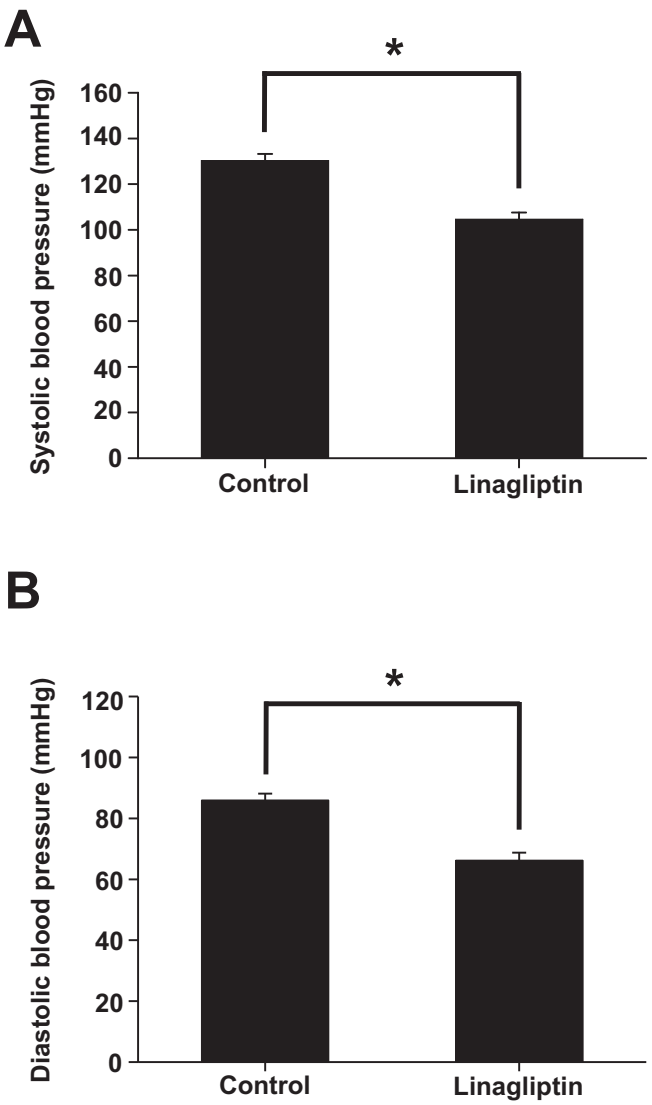

Fig. 9. Effect of linagliptin on blood pressure. (A) Effect of linagliptin on systolic blood pressure. $n = 5$. $^*P < 0.05$ (control vs. linagliptin). (B) Effect of linagliptin on diastolic blood pressure. $n = 5$. $^*P < 0.05$ (control vs. linagliptin). The n means the number of rabbits.

dependent or -independent mechanisms. Endothelium-dependent vasodilation is associated with a variety of vasoactive factors secreted from the endothelium, including NO, endothelium-derived hyperpolarizing factor, and prostacyclin [28]. Therefore, it is important to understand what effect, if any, these factors have on the vasodilatory action of linagliptin. A previous study found that NO inhibited ROCK activity in the rat aorta [29]. Therefore, we investigated whether the vasodilatory effect of linagliptin was mediated by NO released from the endothelium. We found that linagliptin induced vasodilation by inhibiting ROCK activity; however, this effect was not related to endothelium-dependent NO secretion (Figs. 2, 3, and 6). Although the precise mechanism underlying linagliptin-induced inhibition of ROCK activity is not known, it is likely that the drug directly inhibits ROCK activity in vascular smooth muscle.

The cGMP/PKA and cAMP/PKG-dependent signaling pathways are involved in smooth muscle relaxation [30]. Furthermore, cross-talk among NO/cGMP/PKG/ROCK has been reported [31]. In contrast to previous studies, we found no evidence that the cGMP/PKA or cAMP/PKG-dependent signaling pathways contribute to linagliptin-induced vasodilation (Figs. 4 and 5). Activation of the K^+ channels and inhibition of the Ca^{2+} channels expressed in vascular smooth muscle may also significantly affect vasodilation. ROCK is involved in the trafficking of voltage-sensitive K^+ channels [32] and ROCK-dependent suppression of myosin phosphatase activity has been shown to mediate Ca^{2+} sensitization [33]. However, we found no evidence to suggest that linagliptin-induced vasodilation is associated with K^+ and Ca^{2+} channel activity. Although we showed that the inhibition of ROCK activity attenuated linagliptin-induced vasodilation, the fact that the vasodilatory effect was not completely suppressed indicates that other mechanisms are involved in the vasodilatory response to linagliptin. Further study is needed to clarify this issue.

The major limitation of our study was that the vasodilatory effect of linagliptin was observed only at high concentrations: the vasodilatory response was first observed at 10 μM, which is significantly higher than that occurring under physiological conditions. However, overmedication or abuse of linagliptin may increase in therapeutic plasma concentrations of the drug. Although the exact plasma concentration that may result from overmedication or abuse of linagliptin is not known, the blood vessels have high input resistance; therefore, small transient changes in tone have a significant impact on vascular contractility. For this reason, we recommend strict dosing of linagliptin, particularly in patients with hypotension.

In summary, we showed that linagliptin has a vasodilatory effect on the rabbit aorta. Our findings indicate that linagliptin induced vasodilation via the inhibition of ROCK. However, the vasodilatory effect was not associated with the endothelium, cAMP/PKA or cGMP/PKG-dependent signaling pathways, K^+ or Ca^{2+} channels, or SERCA pump.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.01.004.

References

[1] W.T. Cade, Diabetes-related microvascular and macrovascular diseases in the physical therapy setting, Phys. Ther. 88 (2008) 1322–1335.
[2] J. Liu, L. Rutten-Jacobs, M. Liu, et al., Causal impact of type 2 diabetes mellitus on cerebral small vessel disease: a Mendelian randomization analysis, Stroke 49 (2018) 1325–1331.
[3] A. Kibel, K. Selthofer-Relatic, I. Drenjancevic, et al., Coronary microvascular dysfunction in diabetes mellitus, J. Int. Med. Res. 45 (2017) 1901–1929.
[4] N.J. Morrish, S.L. Wang, L.K. Stevens, et al., Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes, Diabetologia 44 (2001) S14–S21.
[5] C. Hampp, V. Borders-Hemphill, D.G. Moeny, et al., Use of antidiabetic drugs in the US, 2003–2012, Diabetes Care 37 (2014) 1367–1374.
[6] M.D. Gorrell, Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders, Clin. Sci. 108 (2005) 277–292.
[7] R.E. Pratley, A. Salsali, Inhibition of DPP-4: a new therapeutic approach for the treatment of type 2 diabetes, Curr. Med. Res. Opin. 23 (2007) 919–931.
[8] D. Baltzis, J.R. Dushay, J. Loader, et al., Effect of linagliptin on vascular function: a randomized, placebo-controlled study, J. Clin. Endocrinol. Metab. 101 (2016) 4205–4213.
[9] F. Shigiyama, N. Kumashiro, M. Miyagi, et al., Linagliptin improves endothelial function in patients with type 2 diabetes: a randomized study of linagliptin effectiveness on endothelial function, J. Diabetes Investig. 8 (2017) 330–340.
[10] S. Kröller-Schön, M. Knorr, M. Hausding, et al., Glucose-independent improvement of vascular dysfunction in experimental sepsis by dipeptidyl-peptidase 4 inhibition, Cardiovasc. Res. 96 (2012) 140–149.
[11] T. Hardigan, Y. Abdul, A. Ergul, Linagliptin reduces effects of ET-1 and TLR2-mediated cerebrovascular hyperreactivity in diabetes, Life Sci. 159 (2016) 90–96.

[12] V. Vellecco, E. Mitidieri, A. Gargiulo, et al., Vascular effects of linagliptin in non-obese diabetic mice are glucose-independent and involve positive modulation of the endothelial nitric oxide synthase (eNOS)/caveolin-1 (CAV-1) pathway, Diabetes Obes. Metab. 18 (2016) 1236–1243.
[13] A.B. Jaffe, A. Hall, Rho GTPases: biochemistry and biology, Annu. Rev. Cell Dev. Biol. 21 (2005) 247–269.
[14] S. Chrissobolis, C.G. Sobey, Evidence that Rho-kinase activity contributes to cerebral vascular tone in vivo and is enhanced during chronic hypertension: comparison with protein kinase C, Circ. Res. 88 (2001) 774–779.
[15] L.A. Calò, A.C. Pessina, RhoA/Rho-kinase pathway: much more than just a modulation of vascular tone. Evidence from studies in humans, J. Hypertens. 25 (2007) 259–264.
[16] K.P. Nunes, C.S. Rigsby, R.C. Webb, RhoA/Rho-kinase and vascular diseases: what is the link? Cell. Mol. Life Sci. 67 (2010) 3823–3836.
[17] T.M. Seasholtz, T. Zhang, M.R. Morissette, et al., Increased expression and activity of RhoA are associated with increased DNA synthesis and reduced p27Kip1 expression in the vasculature of hypertensive rats, Circ. Res. 89 (2001) 488–495.
[18] A. Masumoto, Y. Hirooka, H. Shimokawa, et al., Possible involvement of Rho-kinase in the pathogenesis of hypertension in humans, Hypertension 38 (2001) 1307–1310.
[19] A.F. El-Yazbi, K.S. Abd-Elrahman, ROK and arteriolar myogenic tone generation: molecular evidence in health and disease, Front. Pharmacol. 8 (2017) 87.
[20] S. Sakurada, H. Okamoto, N. Takuwa, et al., Rho activation in excitatory agonist-stimulated vascular smooth muscle, Am. J. Phys. Cell Phys. 281 (2001) 571–578.
[21] C.G. Sobey, Potassium channel function in vascular disease, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 28–38.
[22] Z. Shah, C. Pineda, T. Kampfrath, et al., Acute DPP-4 inhibition modulates vascular tone through GLP-1 independent pathways, Vasc. Pharmacol. 55 (2011) 2–9.
[23] J.R. Wilson, N.J. Brown, H. Nian, et al., Dipeptidyl peptidase-4 inhibition potentiates stimulated growth hormone secretion and vasodilation in women, J. Am. Heart Assoc. 7 (2018) e008000.
[24] Y. Kureishi, S. Kobayashi, M. Amano, et al., Rho-associated kinase directly induces smooth muscle contraction through myosin light chain phosphorylation, J. Biol. Chem. 272 (1997) 12257–12260.
[25] H. Shimokawa, M. Rashid, Development of Rho-kinase inhibitors for cardiovascular medicine, Trends Pharmacol. Sci. 28 (2007) 296–302.
[26] L. Jin, Z. Ying, R.H. Hilgers, et al., Increased RhoA/Rho-kinase signaling mediates spontaneous tone in aorta from angiotensin II-induced hypertensive rats, J. Pharmacol. Exp. Ther. 318 (2006) 288–295.
[27] M. Uehata, T. Ishizaki, H. Satoh, et al., Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension, Nature 389 (1997) 990–994.
[28] A. Sandoo, J.J. van Zanten, G.S. Metsios, et al., The endothelium and its role in regulating vascular tone, Open Cardiovasc. Med. J. 4 (2010) 302–312.
[29] K. Chitaley, R.C. Webb, Nitric oxide induces dilation of rat aorta via inhibition of rho-kinase signaling, Hypertension 39 (2002) 438–442.
[30] T.M. Lincoln, T.L. Cornwell, Towards an understanding of the mechanism of action of cyclic AMP and cyclic GMP in smooth muscle relaxation, Blood Vessels 28 (1991) 129–137.
[31] V. Sauzeau, M. Rolli-Derkinderen, C. Marionneau, et al., RhoA expression is controlled by nitric oxide through cGMP-dependent protein kinase activation, J. Biol. Chem. 278 (2003) 9472–9480.
[32] L. Stirling, M.R. Williams, A.D. Morielli, Dual roles for RHOA/RHO-kinase in the regulated trafficking of a voltage-sensitive potassium channel, Mol. Biol. Cell 20 (2009) 2991–3002.
[33] S. Sakurada, N. Takuwa, N. Sugimoto, et al., Ca2 + -dependent activation of Rho and Rho kinase in membrane depolarization–induced and receptor stimulation–induced vascular smooth muscle contraction, Circ. Res. 93 (2003) 548–556.