ORIGINAL RESEARCH ARTICLE

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The vasorelaxant effect of antidiabetic drug nateglinide via activation of voltage-dependent K$^+$ channels in aortic smooth muscle

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Summary

Aims: We investigated the vasorelaxant effect of nateglinide and its related mechanisms using phenylephrine (Phe)-induced precontracted aortic rings.

Methods: Arterial tone measurement was performed in aortic smooth muscle.

Results: The application of nateglinide induced vasorelaxation in a concentration-dependent manner. Pretreatment with the large-conductance Ca^{2+}-activated K$^+$ (BK$_{Ca}$) channel inhibitor paxilline, the inwardly rectifying K$^+$ (Kir) channel inhibitor Ba^{2+}, and ATP-sensitive K$^+$ (K$_{ATP}$) channel inhibitor glibenclamide did not affect the vasorelaxant effect of nateglinide. However, pretreatment with the voltage-dependent K$^+$ (Kv) channel inhibitor 4-aminopyridine (4-AP) effectively reduced the vasorelaxant effect of nateglinide. Pretreatment with the Ca^{2+} inhibitor nifedipine and the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase inhibitor thapsigargin did not change the vasorelaxant effect of nateglinide. Additionally, the vasorelaxant effect of nateglinide was not altered in the presence of an adenylyl cyclase, a protein kinase A, a guanylyl cyclase, or a protein kinase G inhibitor. The vasorelaxant effect of nateglinide was not affected by the elimination of the endothelium. In addition, pretreatment with a nitric oxide synthase inhibitor, L-NAME, and a small-conductance Ca^{2+}-activated K$^+$ (SK$_{Ca}$) channel inhibitor, apamin, did not change the vasorelaxant effect of nateglinide.

Conclusion: Nateglinide induced vasorelaxation via the activation of the Kv channel independent of other K$^+$ channels, Ca^{2+} channels, intracellular Ca^{2+} ([Ca^{2+}]$_i$), and the endothelium.

KEYWORDS

Aortic smooth muscle, Nateglinide, Vasorelaxation, Voltage-dependent K$^+$ channel

1 | INTRODUCTION

Diabetes mellitus (DM) is one of the most widespread diseases, and the number of individuals diagnosed with DM is increasing. Common symptoms of DM include increased thirst, increased hunger, and more frequent urination. Patients with DM often suffer from numerous complications, such as nephropathy, stroke, heart disease, and diabetic retinopathy.[1-3] Among these, long-term vascular complications are the main cause of death in type 2 DM.[4-6] For this reason, a number of antidiabetic drugs have been developed targeting the dipeptidyl peptidase-4 (DPP-4), gamma isoform of the peroxisome proliferator-activated receptor (PPARγ), alpha-glucosidase, glucagon-like peptide-1 (GLP-1), sodium glucose co-transporter 2 (SGLT2), ATP-dependent potassium (K_{ATP}) channel of pancreatic β-cells.[7] Among these, the phenylalanine derivative nateglinide is a representative meglitinide agent for treating type 2 DM, which stimulates insulin secretion by inhibiting the K_{ATP} channels of pancreatic β-cells.[8] Although nateglinide efficiently controls blood sugar levels, its vasorelaxant effects and mechanisms have been ignored.

Previous studies have demonstrated that four types of K^+ channel are expressed in vascular smooth muscle: voltage-dependent K^+ (Kv), inwardly rectifying K^+ (Kir), large-conductance Ca^{2+}-activated K^+ (BK_{Ca}), and K_{ATP} channels.[9,10] Although most of the K^+ channels are at least partially involved in maintaining vascular tone, Kv channels are regarded as one of the most crucial channels in determining the resting membrane potential and thereby the basal tone of the vessel.[9,11,12] Kv channels are also strongly regulated by intracellular protein kinases including protein kinase C (PKC), protein kinase A (PKA), and protein kinase G (PKG), which are associated with numerous cellular functions.[9] Furthermore, altered Kv channel function is closely related with vascular dysfunction including diabetes, hypertension, hypoxia, and hypertrophy.[13-16] Therefore, the reverse Kv channel function is a potential therapeutic target to recover the vascular function.

Considering the therapeutic efficacy of nateglinide on type 2 DM and the physiological importance of Kv channels in vascular function, it is necessary to elucidate the effect of nateglinide on Kv channels.

Therefore, in this study, we investigated the vasorelaxant effects of nateglinide on rabbit thoracic aorta. Our results showed that nateglinide induced vasorelaxation in a concentration-dependent manner by activating Kv channels in vascular smooth muscle. However, nateglinide-induced vasorelaxation was independent of the cAMP/PKA or the cGMP/PKG signaling pathway, other K^+ channels (BK_{Ca}, K_{ATP}, and Kir), Ca^{2+} channels, intracellular Ca^{2+} ($[Ca^{2+}]_i$), and the endothelium.

2 | MATERIALS AND METHODS

2.1 | Vessel preparation and measurement

Male New Zealand White rabbits (2.0-2.5 kg) were anesthetized by simultaneous injection with heparin (100 U/kg) and sodium pentobarbitone (50 mg/kg) through the ear vein. All animal care and experimental procedures were approved by the Committee for Animal Experiments of Kangwon National University and conform to the NIH
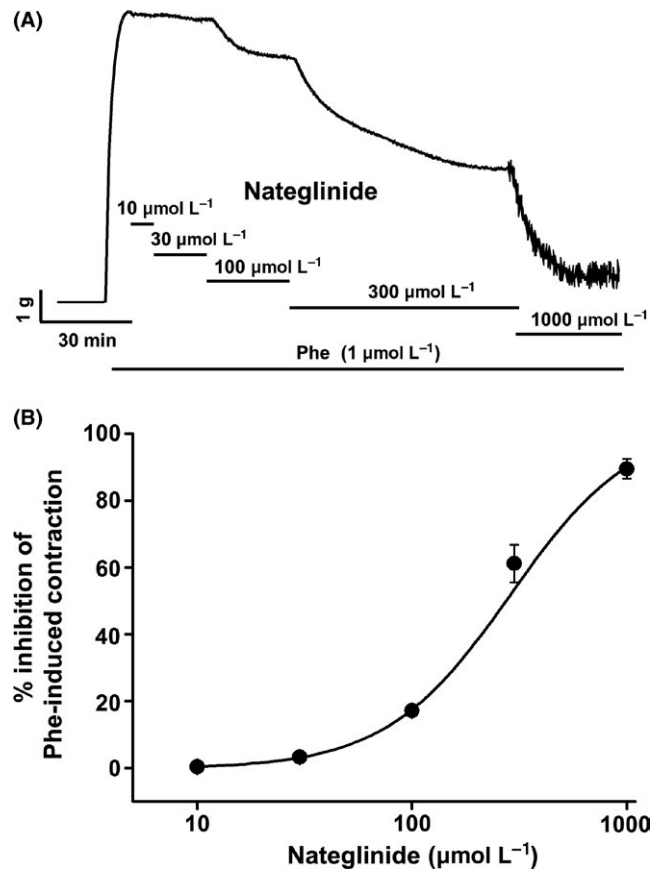
FIGURE 1 The vasorelaxant effect of nateglinide on aortic rings from rabbit. (A) Vasorelaxant effects induced by applying various concentrations of nateglinide (10, 30, 100, 300, and 1000 μmol/L) on Phe-induced precontracted aortic rings. (B) Concentration-dependent curve for the vasorelaxant effect of nateglinide on Phe-induced precontracted aortic rings. All n = 9

guidelines (Guide for the care and use of laboratory animals). The thoracic aorta was rapidly isolated from the heart and cleaned of adipose and connective tissue in normal Tyrode's solution, visualized under a stereomicroscope. The purified aorta was cut into rings of ~10 mm in length, and the rings were incubated in an organ bath containing oxygenated (95% O_2 and 5% CO_2) physiological salt solution (PSS). The arteries were maintained at a resting tension of 1 g for 2 hours at 37°C. Endothelium-denuded arteries were prepared by intraluminal injection of air bubbles for 10 minutes. Arterial viability was tested by applying high K^+ (80 mmol/L)-PSS before starting the experiments.

2.2 | Solutions and chemicals

The normal Tyrode's solution containing (mmol/L) KCl 5.4, NaCl 143, $CaCl_2$ 1.8, NaH_2PO_4 0.33, HEPES 5, $MgCl_2$ 0.5, and Glucose 15 adjusted to pH 7.4 with NaOH. PSS containing (mmol/L) KCl 4.7, NaCl 120, $CaCl_2$ 1.8, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, and Glucose 15 adjusted to pH 7.4 with NaOH. Phenylephrine (Phe), 4-aminopyridine (4-AP), and $BaCl_2$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in distilled water. Nateglinide, acetylcholine, paxilline, glibenclamide, SQ 22536,

ODQ, KT 5720, KT 5823, L-NAME, apamin, nifedipine, thapsigargin, DPO-1, and guangxitoxin were purchased from Tocris Cookson (Ellisville, MO, USA) and dissolved in dimethyl sulfoxide (DMSO) or distilled water.

2.3 | Data analysis

Data were analyzed using Origin v.7.0 software (Microcal Software, Inc., Northampton, MA, USA). The results are presented as means ± standard error of the mean (SEM). Student's t tests were applied to evaluate statistical significance. A value of $P < .05$ was regarded as statistically significant.

3 | RESULTS

3.1 | The effect of nateglinide on Phe-induced precontracted aortic rings

The vasorelaxant effects of nateglinide were investigated using Phe-induced precontracted aortic rings. As shown in Figure 1A,

nateglinide induced vasorelaxation in a concentration-dependent fashion. For example, application of 300 µmol/L nateglinide induced 61.18% vasorelaxation on Phe-induced precontracted aortic rings (Figure 1B). The application of 1000 µmol/L nateglinide induced vasorelaxation almost to the base line level (before precontraction with Phe). The higher concentration of 1000 µmol/L nateglinide slightly induced further vasorelaxation; however, the degree of vasorelaxation did not statistically differ when applying 1000 µmol/L nateglinide.

3.2 | Effect of K^+ channel inhibitors on nateglinide-induced vasorelaxation

To test the involvement of the K^+ channel on the vasorelaxant effect of nateglinide, we pretreated four types of K^+ channel inhibitors on nateglinide-induced vasorelaxation. As shown in Figure 2A, application of the BK_{Ca} channel inhibitor paxilline (10 µmol/L) did not change the arterial tone of Phe-induced precontracted aortic rings. Furthermore, pretreatment with paxilline did not affect the vasorelaxant effect of nateglinide (Figure 2B). Application of the Kir channel
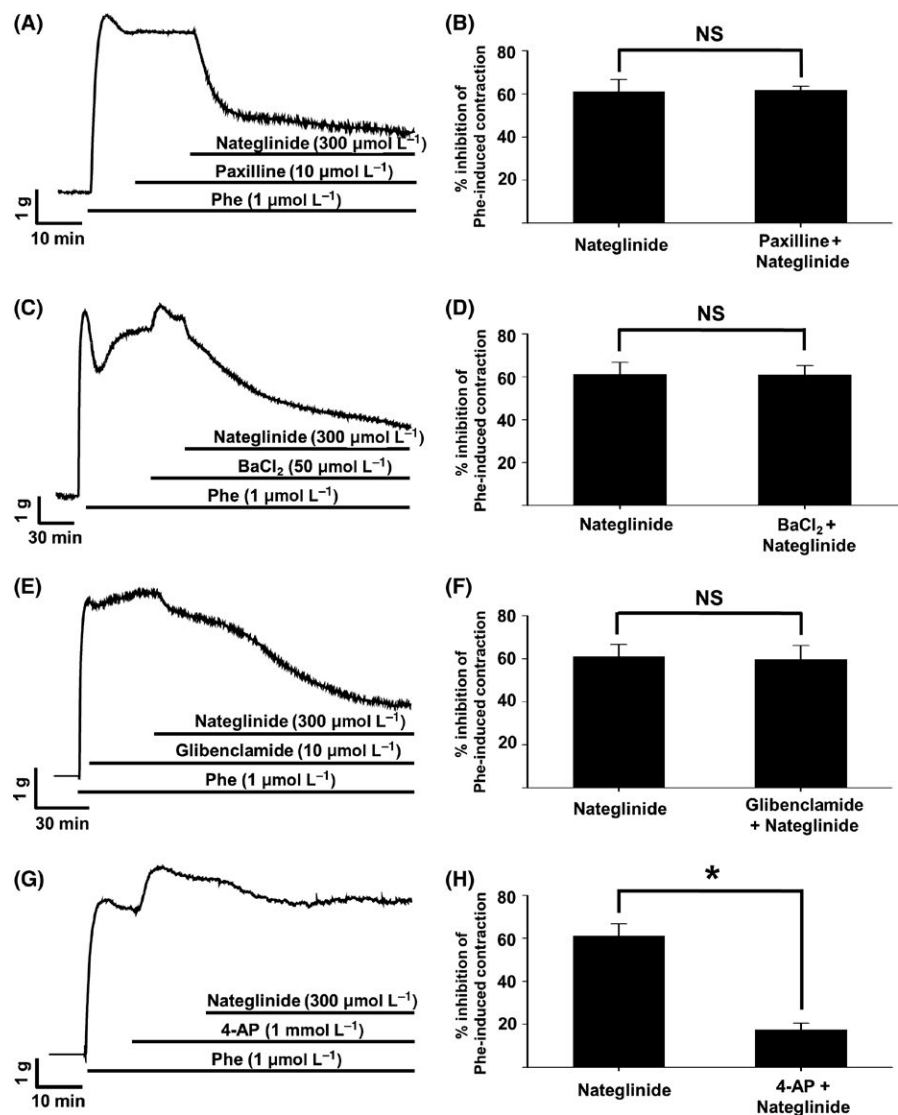
FIGURE 2 Involvement of K^+ channels on nateglinide-induced vasorelaxation. (A) Effect of the BK_{Ca} channel inhibitor paxilline on nateglinide-induced vasorelaxation. (B) Summary of the effects of paxilline on nateglinide-induced vasorelaxation. n = 4. NS = not significant (nateglinide vs paxilline + nateglinide, by Student's t tests). (C) Effect of the Kir channel inhibitor Ba^{2+} on nateglinide-induced vasorelaxation. (D) Summary of the effects of Ba^{2+} on nateglinide-induced vasorelaxation. n = 5. NS = not significant (nateglinide vs Ba^{2+} + nateglinide, by Student's t tests). (E) Effect of the K_{ATP} channel inhibitor glibenclamide on nateglinide-induced vasorelaxation. (F) Summary of the effects of glibenclamide on nateglinide-induced vasorelaxation. n = 5. NS = not significant (nateglinide vs glibenclamide + nateglinide, by Student's t tests). (G) Effect of the Kv channel inhibitor 4-AP on nateglinide-induced vasorelaxation. (H) Summary of the effects of 4-AP on nateglinide-induced vasorelaxation. n = 6. *$P < .05$ (nateglinide vs 4-AP + nateglinide, by Student's t tests)

inhibitor Ba^{2+} induced further vasoconstriction on Phe-induced pre-contracted aortic rings (Figure 2C). However, pretreatment with Ba^{2+} did not alter the vasorelaxant effect of nateglinide (Figure 2D). We also tested the effect of the K_{ATP} channel inhibitor glibenclamide on nateglinide-induced vasorelaxation. As shown in Figure 2E,F, the vasorelaxant effect of nateglinide was not affected by pretreatment with glibenclamide. Similar to the results observed with Ba^{2+}, pretreatment with the Kv channel inhibitor 4-AP slightly induced further vasoconstriction (Figure 2G). However, pretreatment with 4-AP effectively inhibited the vasorelaxant effect of nateglinide (Figure 2H). These results suggest that the vasorelaxant effect of nateglinide on aortic smooth muscle was closely related to the activation of the Kv channel, not the BK_{Ca}, Kir, or K_{ATP} channels.

3.3 | Effect of Ca^{2+} channel inhibitor and a sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitor on nateglinide-induced vasorelaxation

To investigate the involvement of Ca^{2+} influx by activation of Ca^{2+} channels on nateglinide-induced vasorelaxation, we pretreated the Phe-induced precontracted aortic rings with the *L*-type Ca^{2+} channel inhibitor nifedipine (10 μmol/L). Pretreatment with nifedipine had no effect on the maximal Phe-induced vasoconstriction; Phe-induced vasoconstriction in control and in presence of nifedipine were 5.49 ± 0.42 (n = 6) and 5.28 ± 0.38 g (n = 6), respectively. Figure 3A,B shows that the blockade of the Ca^{2+} channel by nifedipine did not change the vasorelaxant effect of nateglinide. In addition, we also used a SERCA inhibitor to deplete $[Ca^{2+}]_i$ on nateglinide-induced vasorelaxation. Pretreatment with SERCA inhibitor, thapsigargin (1 μmol/L), also had no significant impact on the maximal Phe-induced vasoconstriction; Phe-induced vasoconstriction in control and in presence of thapsigargin were 5.62 ± 0.55 (n = 4) and 5.51 ± 0.60 g (n = 4), respectively. As shown in Figure 3C,D, pretreatment with thapsigargin did not alter the vasorelaxant effect of nateglinide. These results implied that the vasorelaxant effect of nateglinide was not related to the Ca^{2+} channels or $[Ca^{2+}]_i$.

3.4 | Involvement of adenylyl cyclase and PKA on nateglinide-induced vasorelaxation

To determine whether nateglinide-induced vasorelaxation was mediated by the activation of adenylyl cyclase and thereby PKA, we pretreated the Phe-induced precontracted aortic rings with the adenylyl cyclase inhibitor SQ 22536 (50 μmol/L). Pretreatment with SQ 22536 did not alter the maximal Phe-induced vasoconstriction (control, 5.23 ± 0.37; presence of SQ 22536, 5.66 ± 0.62 g, all n = 5). As shown in Figure 4A,B, adenylyl cyclase inhibition by pretreatment of SQ 22536 had no effect on nateglinide-induced vasorelaxation. Furthermore, pretreatment with PKA inhibitor, KT 5720 (1 μmol/L), did not change the maximal Phe-induced vasoconstriction (control, 5.33 ± 0.40; presence of KT 5720, 5.07 ± 0.52 g, all n = 5) and did not reduce the vasorelaxant effect of nateglinide (Figure 4C,D). These

results suggest that the vasorelaxant effect of nateglinide did not involve the accumulation of cAMP by stimulation of adenylyl cyclase and therefore the activation of PKA.

3.5 | Involvement of guanylyl cyclase and PKG on nateglinide-induced vasorelaxation

To further determine whether the vasorelaxant effect of nateglinide was related to the activation of guanylyl cyclase and PKG, we applied the guanylyl cyclase inhibitor, ODQ (1 μmol/L) to Phe-induced precontracted aortic rings. No changes in maximal constriction we observed after pretreatment with ODQ (control, 5.52 ± 0.54; presence of ODQ, 5.81 ± 0.58 g, all n = 6). As shown in Figure 5A,B, the application of ODQ had no significant effect on nateglinide-induced vasorelaxation. Additional experiments revealed that pretreatment with PKG inhibitor, KT 5823 (1 μmol/L), also did not alter the maximal Phe-induced vasoconstriction (control, 5.29 ± 0.39; presence of KT 5823, 5.17 ± 0.32 g, all n = 7) nor the vasorelaxant effect of nateglinide (Figures 5C,D). From these results, we concluded that the vasorelaxant effect of nateglinide did not involve in the activation of guanylyl cyclase and PKG.

3.6 | Endothelium-dependency of nateglinide-induced vasorelaxation

To confirm that the vasorelaxant effect of nateglinide was related to the endothelial-dependent mechanism, we investigated the vasorelaxant effect of nateglinide on endothelium-denuded aortic rings. Figure 6A shows that nateglinide induced vasorelaxation in a concentration-dependent manner on endothelium-denuded aortic rings. However, the degree of vasorelaxation did not differ between the endothelium-intact and endothelium-denuded aortic rings (Figure 6B). These results strongly suggested that the vasorelaxant effect of nateglinide was independent of the endothelium.

To confirm that the endothelium was not involved in mediating nateglinide-induced vasorelaxation, we pretreated the sample with a nitric oxide (NO) synthase inhibitor, L-NAME (100 μmol/L), or a small-conductance Ca^{2+}-activated K^+ channel (SK_{Ca}) inhibitor, apamin (1 μmol/L), on the endothelium-intact aortic rings. The application of L-NAME induced further constriction in the aortic rings. However, the vasorelaxant effect of nateglinide was not affected by pretreatment with L-NAME (Figure 7A,B). Furthermore, application of apamin did not alter the vasorelaxant effect of nateglinide (Figure 7C,D). Therefore, we concluded that nateglinide-induced vasorelaxation occurred independently of the endothelium.
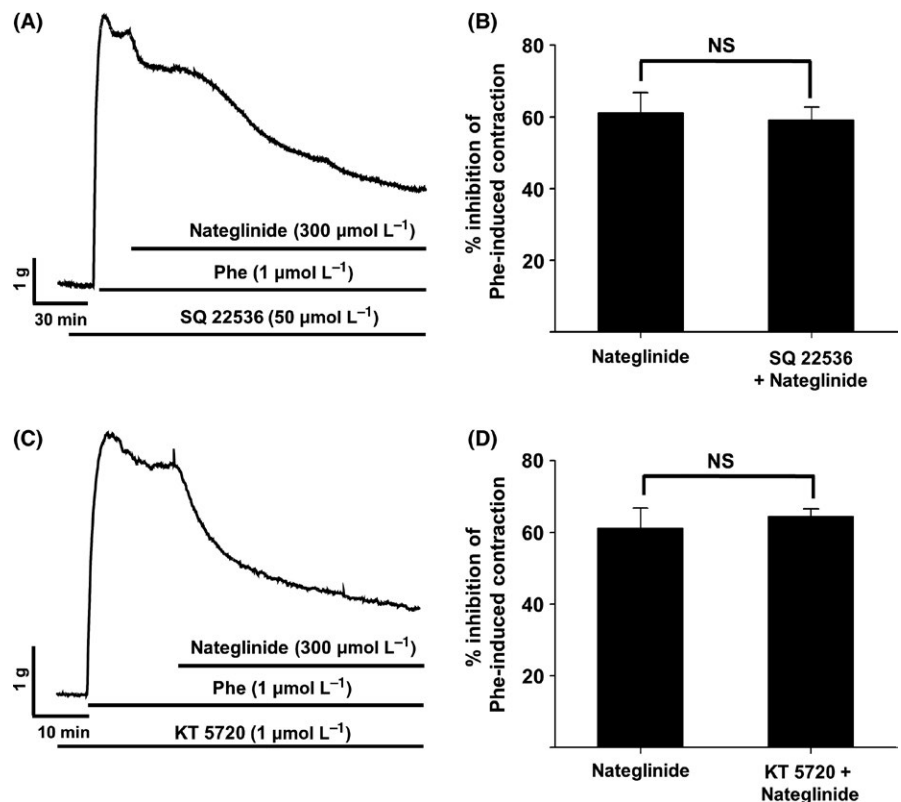
3.7 | Nateglinide-induced vasorelaxation in the presence of a Kv1.5 or Kv2.1 subtype inhibitor

As illustrated in Figure 2, the vasorelaxant effect of nateglinide was mediated by the activation of Kv channels. Therefore, to test the involvement of Kv subtypes in nateglinide-induced vasorelaxation, we recorded the vasorelaxant effect of nateglinide in the

FIGURE 3 Involvement of Ca^{2+} channels and intracellular Ca^{2+} on the vasorelaxant effect of nateglinide. (A) Effect of the *L*-type Ca^{2+} channel inhibitor nifedipine on nateglinide-induced vasorelaxation. (B) Summary of the effects of nifedipine on nateglinide-induced vasorelaxation. n = 6. NS = not significant (nateglinide vs nifedipine + nateglinide, by Student's *t* tests). (C) Effect of the SERCA inhibitor thapsigargin on nateglinide-induced vasorelaxation. (D) Summary of the effects of thapsigargin on nateglinide-induced vasorelaxation. n = 4. NS = not significant (nateglinide vs thapsigargin + nateglinide, by Student's *t* tests)
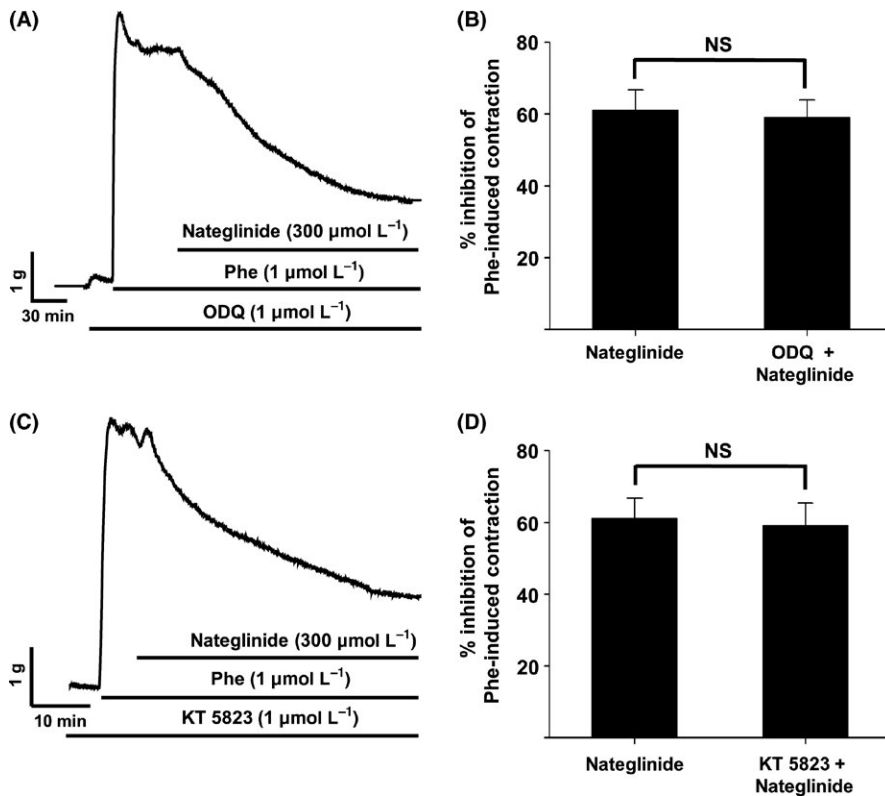
FIGURE 4 Effects of an adenylyl cyclase and a PKA inhibitor on nateglinide-induced vasorelaxation. (A) Influence of the adenylyl cyclase inhibitor SQ 22536 on nateglinide-induced vasorelaxation. (B) Summary of the effects of SQ 22536 on nateglinide-induced vasorelaxation. n = 5. NS = not significant (nateglinide vs SQ 22536 + nateglinide, by Student's *t* tests). (C) Influence of the PKA inhibitor KT 5720 on nateglinide-induced vasorelaxation. (D) Summary of the effects of KT 5720 on nateglinide-induced vasorelaxation. n = 5. NS = not significant (nateglinide vs KT 5720 + nateglinide, by Student's *t* tests)

presence of a Kv1.5 or Kv2.1 subtype inhibitor. Kv1.5 and/or Kv2.1 are known as major subtypes in vascular smooth muscle[10,15] and the inhibitors for Kv1.5 and Kv2.1 subtypes are among the best characterized for Kv channel modulation. As shown in Figure 8A,B, pretreatment with a Kv1.5 channel inhibitor, DPO-1 (1 μmol/L), did not alter the vasorelaxant effect of nateglinide. Similar to the results

FIGURE 5 Effects of a guanylyl cyclase and a PKG inhibitor on nateglinide-induced vasorelaxation. (A) Influence of the guanylyl cyclase inhibitor ODQ on nateglinide-induced vasorelaxation. (B) Summary of the effects of ODQ on nateglinide-induced vasorelaxation. n = 6. NS = not significant (nateglinide vs ODQ + nateglinide, by Student's *t* tests). (C) Influence of the PKG inhibitor KT 5823 on nateglinide-induced vasorelaxation. (D) Summary of the effects of KT 5823 on nateglinide-induced vasorelaxation. n = 7. NS = not significant (nateglinide vs KT 5823 + nateglinide, by Student's *t* tests)

observed with DPO-1, application of the Kv2.1 channel inhibitor guangxitoxin (100 nmol/L) did not alter the vasorelaxant effect of nateglinide (Figure 8C,D). From these results, we concluded that nateglinide-induced vasorelaxation was not related to activation of the Kv1.5 or Kv2.1 subtype.

4 | DISCUSSION

Our study demonstrated the nateglinide-induced vasorelaxation of the rabbit thoracic aorta in a concentration-dependent manner via activation of the Kv channel expressed in smooth muscle. However, this effect did not involve the BK_{Ca}, K_{ATP}, Kir, Ca^{2+} channels, or $[Ca^{2+}]_i$, and was independent of the endothelium.

In the past few decades, research into diabetes has been concerned with controlling the blood sugar levels. Considering that 90% of diabetics are diagnosed with type 2 DM, most research has focused on the development of type 2 DM antidiabetic therapies.[1-3] The phenylalanine derivative nateglinide is an effective oral hypoglycemic agent for treating type 2 DM, which stimulates insulin secretion by inhibiting the K_{ATP} channel of the pancreatic β-islet cells.[8] Compared with other traditional antidiabetic drugs, nateglinide has demonstrated greater safety and efficacy in treating type 2 DM. In fact, the first and second generations of sulfonylureas effectively lowered blood sugar levels by increasing the secretion of insulin; however, these agents also caused hyperinsulinemia and prolonged hypoglycemia.[17] PPARγ activators, such as troglitazone and pioglitazone, can cause hepatotoxicity,

bladder cancer, or congestive heart failure.[18,19] Although nateglinide causes common side effects similar to other antidiabetic drugs, these side effects are acceptable and nonfatal.

A previous study demonstrated that nateglinide decreased the systemic blood pressure of patients with type 2 DM.[20] Although the authors did not provide any mechanistic evidence, they speculated that the decrease in the systemic blood pressure by nateglinide could be attributed to the improvement of endothelial dysfunction. However, our study strongly suggested that the vasorelaxant effect of nateglinide was independent of the endothelium, but was associated with ion channel activation, specifically the Kv channel expressed in smooth muscle. Therefore, activation of Kv channels by nateglinide induced vasorelaxation and could thereby decrease the systemic blood pressure in patients with type 2 DM.

The present study showed that Kv channel inhibition, but not K_{ATP}, Kir, or BK_{Ca} channel inhibition, greatly reduced nateglinide-induced vasorelaxation. This suggests that nateglinide effectively induced the activation of the Kv channel (Figure 2). Considering the physiological and pathological relevance of Kv channels as described in the Introduction, detailed information for the involvement of specific Kv subtypes on nateglinide-induced vasorelaxation will be required. Regarding the Kv subtypes expressed in the vascular smooth muscle, the molecular identity of the Kv channels is still controversial. Furthermore, studies on the molecular identity of the Kv channels have been mainly performed with tissue from rat or mouse, not rabbit. For this reason, it could be difficult to identify which subtypes are expressed in the rabbit aorta. However, the available studies indicate
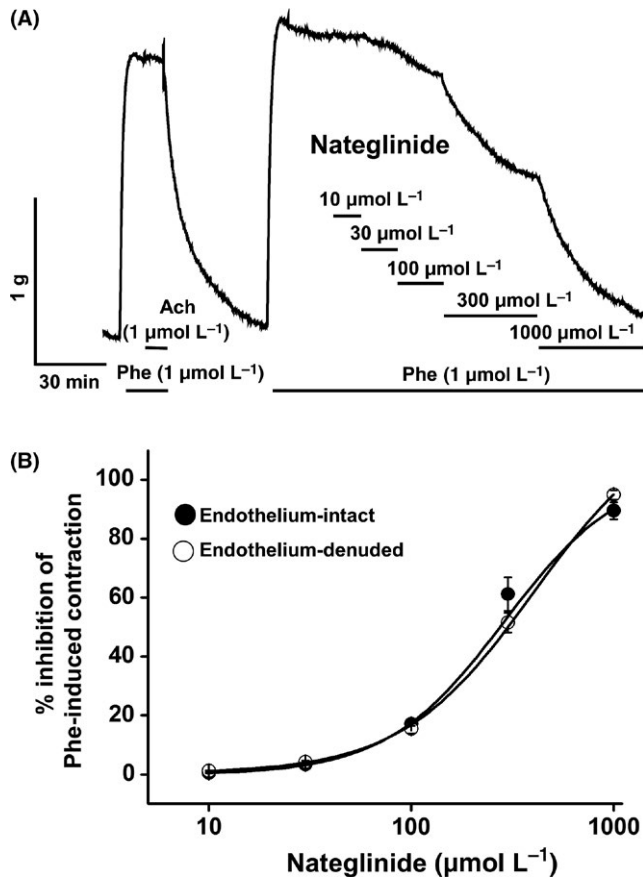
FIGURE 6 Comparison of nateglinide-induced changes in vascular tone in endothelium-intact and endothelium-denuded aortic rings. (A) The vasorelaxant effects of nateglinide as determined by applying various concentrations of nateglinide to endothelium-denuded aortic rings. (B) The concentration-dependent curves for the nateglinide-induced vasorelaxation on endothelium-intact and endothelium-denuded aortic rings. n = 4

that Kv1.2, Kv1.4, Kv1.5, Kv2.1, and Kv9.3 subtypes have been detected in common vascular smooth muscle. Specifically, most previous studies agreed that Kv1.5 and Kv2.1 subtypes are the major subtypes in vascular smooth muscle.[10,15,21,22] Therefore, to further evaluate the specific Kv subtypes involved in nateglinide-induced vasorelaxation, a Kv1.5 or Kv2.1 subtype inhibitor was applied before treatment with nateglinide. However, our results indicated that neither the Kv1.5 nor Kv2.1 subtype inhibitor affected the nateglinide-induced vasorelaxation (Figure 8). These results suggest that nateglinide-induced vasorelaxation was not due to the activation of Kv1.5 or Kv2.1 channels; alternatively, nateglinide-induced vasorelaxation might be necessary for the activation of several Kv subtypes simultaneously. This issue should be addressed in future studies.
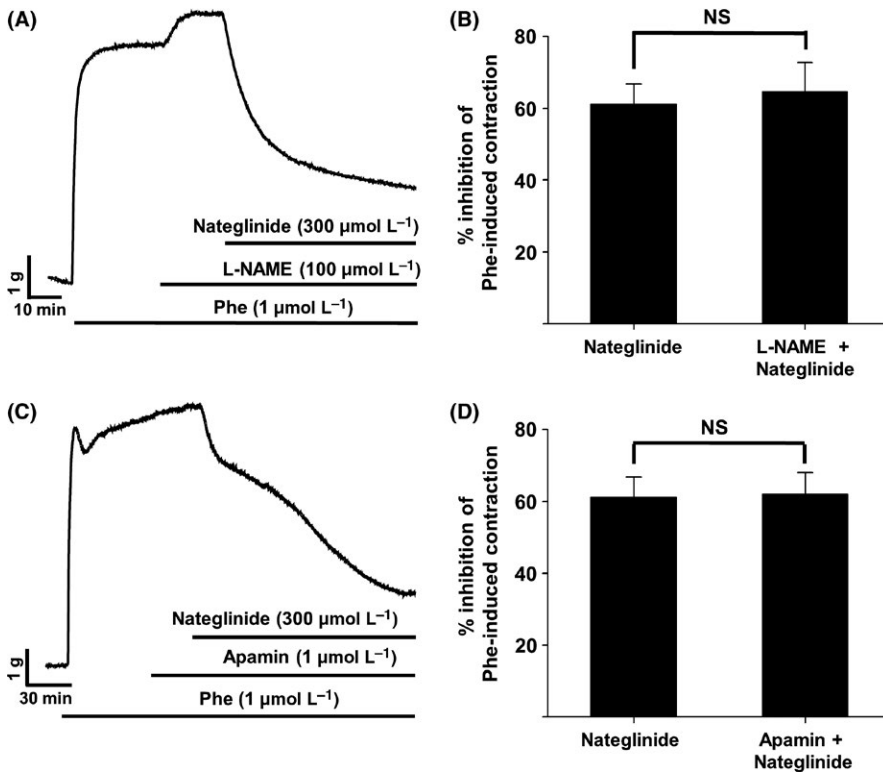
Numerous reports have suggested that Kv channels in the vascular smooth muscle are strongly regulated by intracellular protein kinases, such as PKC, PKA, and PKG. In general, a number of vasodilators activate adenylyl cyclase and thereby PKA, and/or activate guanylyl cyclase, and consequently PKG. Activation of PKA and/or PKG results in the activation of vascular K$^+$ channels, which induces vasorelaxation.[10,23,24] Therefore, activation of Kv channels by nateglinide could

be involved in the activation of PKA and/or PKG. However, our results suggested that the application of adenylyl cyclase, guanylyl cyclase, PKA inhibitor, and PKG inhibitor did not affect the vasorelaxant effect of nateglinide. Furthermore, application of a MEK inhibitor (PD 98059) and a ROCK inhibitor (Y-27632) did not affect the vasorelaxant effect of nateglinide (data not shown).Therefore, we concluded that PKA- and PKG-dependent signaling pathways were not involved in the vasorelaxant effect of nateglinide (Figures 4 and 5).
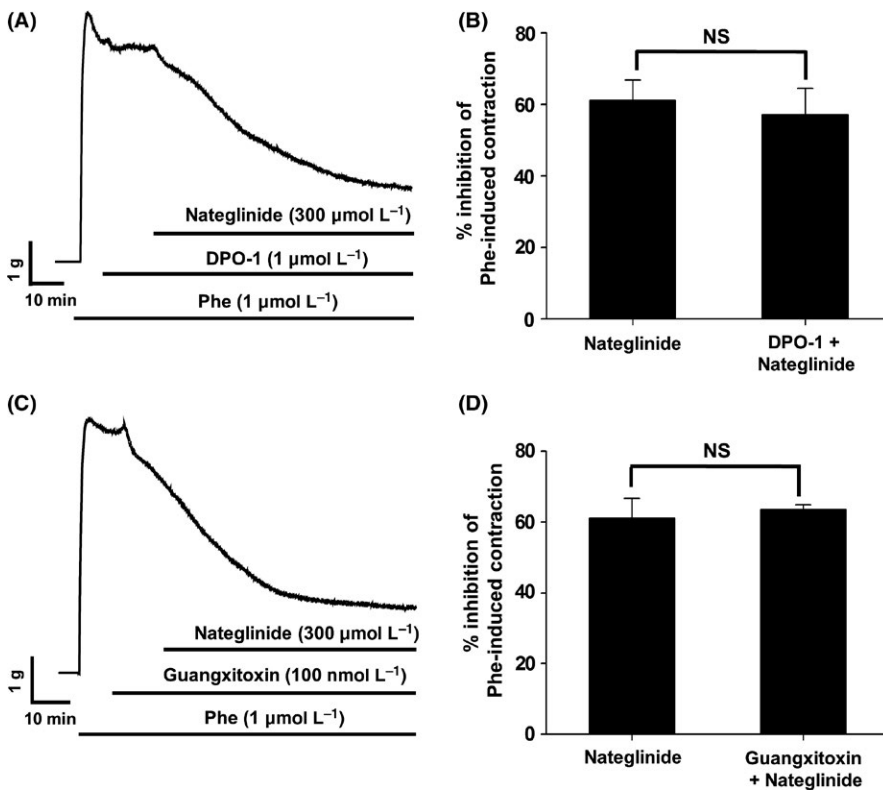
Many cases of vasorelaxation induced by drugs occur through endothelium-dependent mechanisms. In fact, nateglinide reportedly abrogates endothelial dysfunction by decreasing the levels of glucose.[25-28] However, our results clearly demonstrated that the vasorelaxant effect of nateglinide did not differ in endothelium-intact and endothelium-denuded arteries. Furthermore, application of NO synthase inhibitor L-NAME and SK$_{Ca}$ inhibitor apamin did not alter the vasorelaxant effect of nateglinide (Figures 6 and 7). Although our results could not address the exact mechanisms involving in the activation of Kv channels by nateglinide, taken together our results show that nateglinide could interact with Kv channel directly without involvement of intracellular signaling pathways.

Several previous studies have shown that cannabinoid cause vasorelaxation in some animals and human arterial beds by activation of cannabinoid receptor 1 (CB1), and/or cannabinoid receptor 2 (CB2).[29] For example, arachidonylcyclopropylamide (ACPA), a selective and potent CB1 agonist, caused vasorelaxation of aortic rings through the activation of K$_{Ca}$1.1 and the inhibition of Ca$_v$1.2.[30] In addition, cannabidiol induced vasorelaxation of human mesenteric arteries in endothelium-dependent manner through the activation of CB1.[31] For these reasons, the therapeutic potential and effect of cannabidiol have been more widely explored in vascular dysfunctions including type 1 and type 2 diabetes. In fact, cannabidiol improved vasorelaxant responses of femoral arteries in Zucker diabetic fatty (ZDF) rats (type 2 diabetic models) through the activation of CB2, cyclooxygenase, and superoxide dismutase, but this effect was endothelium independent.[32] More recently, the same group suggested that short-term in vivo treatment with cannabidiol was related with improvements in endothelium-dependent vasorelaxation in mesenteric arteries from ZDF rats.[33] In the present study, we did not perform the experiments regarding the association between nateglinide-induced vasorelaxation and CB receptor activation. However, as CB receptors are closely related with vasorelaxation, there is a possibility that CB receptor activation is involved in the vasorelaxant effect of nateglinide. We will address this in future studies.

Clinically, nateglinide, as an oral antidiabetic drug, is prescribed in fixed doses (120 mg) before every meal. Nateglinide has been shown to be rapidly absorbed, with peak blood and plasma concentrations at ~1 hour post dose (~18 µmol/L).[34] Our present study showed that nateglinide predominantly induced vasorelaxation at higher concentrations (>30 µmol/L), which is greater than the peak concentration of nateglinide in the blood and cannot be reached under normal physiological conditions. However, an overdose of nateglinide could raise the levels of nateglinide to greater than 18 µmol/L. Furthermore, the vasorelaxant effect of nateglinide

(A)

Nateglinide (300 µmol L⁻¹)

L-NAME (100 µmol L⁻¹)

Phe (1 µmol L⁻¹)

1 g

10 min

(B)

NS

% inhibition of Phe-induced contraction

80

60

40

20

Nateglinide   L-NAME + Nateglinide

(C)

Nateglinide (300 µmol L⁻¹)

Apamin (1 µmol L⁻¹)

Phe (1 µmol L⁻¹)

1 g

30 min

(D)

NS

% inhibition of Phe-induced contraction

80

60

40

20

Nateglinide   Apamin + Nateglinide

FIGURE 7 The effects of the NO synthase inhibitor L-NAME and SK_{Ca} inhibitor apamin on nateglinide-induced vasorelaxation in endothelium-intact aortic rings (A) Effect of the NO synthase inhibitor L-NAME on nateglinide-induced vasorelaxation. (B) Summary of the effects of L-NAME on nateglinide-induced vasorelaxation. n = 5. NS = not significant (nateglinide vs L-NAME + nateglinide, by Student's t tests). (C) Effect of the SK_{Ca} channel inhibitor apamin on nateglinide-induced vasorelaxation. (D) Summary of the effects of apamin on nateglinide-induced vasorelaxation. n = 5. NS = not significant (nateglinide vs apamin + nateglinide, by Student's t tests)

(A)

Nateglinide (300 µmol L⁻¹)

DPO-1 (1 µmol L⁻¹)

Phe (1 µmol L⁻¹)

1 g

10 min

(B)

NS

% inhibition of Phe-induced contraction

80

60

40

20

0

Nateglinide   DPO-1 + Nateglinide

(C)

Nateglinide (300 µmol L⁻¹)

Guangxitoxin (100 nmol L⁻¹)

Phe (1 µmol L⁻¹)

1 g

10 min

(D)

NS

% inhibition of Phe-induced contraction

80

60

40

20

0

Nateglinide   Guangxitoxin + Nateglinide

FIGURE 8 Effects of Kv1.5 and Kv2.1 subtype inhibitors on nateglinide-induced vasorelaxation. (A) Vasorelaxant effects of nateglinide in the presence of the Kv1.5 subtype inhibitor DPO-1 on Phe-induced precontracted aortic rings. (B) Summary of the effects of DPO-1 on nateglinide-induced vasorelaxation. n = 4. NS = not significant (nateglinide vs DPO-1 + nateglinide, by Student's t tests). (C) Vasorelaxant effects of nateglinide in the presence of the Kv2.1 subtype inhibitor guangxitoxin on Phe-induced precontracted aortic rings. (D) Summary of the effects of guangxitoxin on nateglinide-induced vasorelaxation. n = 5. NS = not significant (nateglinide vs guangxitoxin + nateglinide, by Student's t tests)

at lower concentrations (<30 µmol/L) was also effective, despite a lesser degree of vasorelaxation (~5%). Considering that arterial smooth muscle has a very high input resistance, small changes in vascular tone could change the blood pressure and blood flow. Therefore, tight control of nateglinide dosing is needed, especially in hypotensive patients. In diabetic patients with hypertension, nateglinide not only improved the blood sugar level but also helped to decrease blood pressure.

In the present study, we demonstrated the vasorelaxant effect of nateglinide on rabbit aorta. Our results showed that nateglinide induced vasorelaxation by activating the Kv channel. However, other K^+ channels, Ca^{2+} channels, $[Ca^{2+}]_i$, PKA/PKG-related signaling pathways, and the endothelium were not involved in nateglinide-induced vasorelaxation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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