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The anti-diabetic drug repaglinide induces vasorelaxation via activation of PKA and PKG in aortic smooth muscle

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ABSTRACT

We investigated the vasorelaxant effect of repaglinide and its related signaling pathways using phenylephrine (Phe)-induced pre-contracted aortic rings. Repaglinide induced vasorelaxation in a concentration-dependent manner. The repaglinide-induced vasorelaxation was not affected by removal of the endothelium. In addition, application of a nitric oxide synthase inhibitor (L-NAME) and a small-conductance Ca^{2+}-activated K^+ (SK_{Ca}) channel inhibitor (apamin) did not alter the vasorelaxant effect of repaglinide on endothelium-intact arteries. Pretreatment with an adenylyl cyclase inhibitor (SQ 22536) or a PKA inhibitor (KT 5720) effectively reduced repaglinide-induced vasorelaxation. Also, pretreatment with a guanylyl cyclase inhibitor (ODQ) or a PKG inhibitor (KT 5823) inhibited repaglinide-induced vasorelaxation. However, pretreatment with a voltage-dependent K^+ (Kv) channel inhibitor (4-AP), ATP-sensitive K^+ (K_{ATP}) channel inhibitor (glibenclamide), large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel inhibitor (paxilline), or the inwardly rectifying K^+ (Kir) channel inhibitor (Ba^{2+}) did not affect the vasorelaxant effect of repaglinide. Furthermore, pretreatment with a Ca^{2+} inhibitor (nifedipine) and a sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitor (thapsigargin) did not affect the vasorelaxant effect of repaglinide. The vasorelaxant effect of repaglinide was not affected by elevated glucose (50 mM). Based on these results, we conclude that repaglinide induces vasorelaxation via activation of adenylyl cyclase/PKA and guanylyl cyclase/PKG signaling pathways independently of the endothelium, K^+ channels, Ca^{2+} channels, and intracellular Ca^{2+} ($[Ca^{2+}]_i$).

1. Introduction

Diabetes mellitus (DM), the most common metabolic disease, is characterized by high blood glucose levels. Specifically, type 2 DM comprises 90% of all DM cases and causes several complications, such as heart disease, cardiovascular disease, nephropathy, stroke and retinopathy [2,29,32]. Among these, long-term vascular complications are the main cause of morbidity and mortality in type 2 DM patients [29].

To date, numerous anti-diabetic drugs have been developed. Among these, metformin is used as the first-line drug to treat type 2 DM. The molecular action of metformin is related mainly to the activation of AMP-activated protein kinase (AMPK) [33]. Inhibitors of the ATP-sensitive potassium channel of pancreatic β-cells, such as sulfonylureas and meglitinides, are also regarded as effective drugs for the treatment of diabetes [1,15]. Other types of anti-diabetic drugs, such as alpha-glucosidase inhibitors, thiazolidinediones, glucagon like peptide-1 (GLP-1) agonists, dipeptidyl peptidase-4 (DPP-4) inhibitors, and sodium glucose co-transporter 2 (SGLT2) inhibitors, have also been developed [4,8]. Repaglinide, one of the meglitinide drugs, is used widely for the treatment of type 2 diabetes [28]. Similar to sulfonylurea, repaglinide also stimulates insulin secretion via inhibition of the ATP-dependent potassium channels of pancreatic β-cells, but at a different binding site [10]. Previous studies have demonstrated that repaglinide could effectively lower blood sugar, and glycemic normalization improved both the microcirculation and mild diastolic dysfunction [13,26]. Based on these studies, it is possible that repaglinide is able to induce vasorelaxation. The primary aim of the present study was, therefore, to investigate the

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vasorelaxant effect of repaglinide on aortic rings and its related signaling mechanisms.

Previous reports have determined that second messengers, such as cyclic AMP (cAMP), cyclic GMP (cGMP), inositol triphosphate, diacylglycerol, and calcium, are one of the initiating components of intracellular signal transduction, which is closely related to many cellular physiological activities [7,16,17]. Among the second messengers, intracellular levels of cAMP and cGMP are closely related to vascular tone [19,24]. In general, vasodilator drugs and endogenous vasodilators increase cAMP and/or cGMP levels via activation of adenylyl cyclase and/or guanylyl cyclase, and thereby subsequent activation of AMP-dependent protein kinase (PKA) and/or cGMP-dependent protein kinase (PKG) [31]. Both of these second messengers appear to reduce the concentration of intracellular Ca^{2+} in vascular smooth muscle cells, and therefore induce vasorelaxation [19,24].

Considering the efficacy of repaglinide as an anti-diabetic drug and the physiological relevance of PKA- and PKG-related signaling on vasorelaxation, we sought to elucidate whether the vasorelaxant effect of repaglinide was associated with the cAMP/PKA and/or cGMP/PKG signaling pathway.

In the current study, we investigated the vasorelaxant effects of repaglinide and its related signaling pathways using rabbit aortic smooth muscle. The results clearly demonstrate that repaglinide induces vasorelaxation via activation of adenylyl cyclase/PKA and guanylyl cyclase/PKG signaling pathways. However, repaglinide-induced vasorelaxation is independent of endothelium, K^+ channels, Ca^{2+} channels, and intracellular Ca^{2+} ($[Ca^{2+}]_i$).

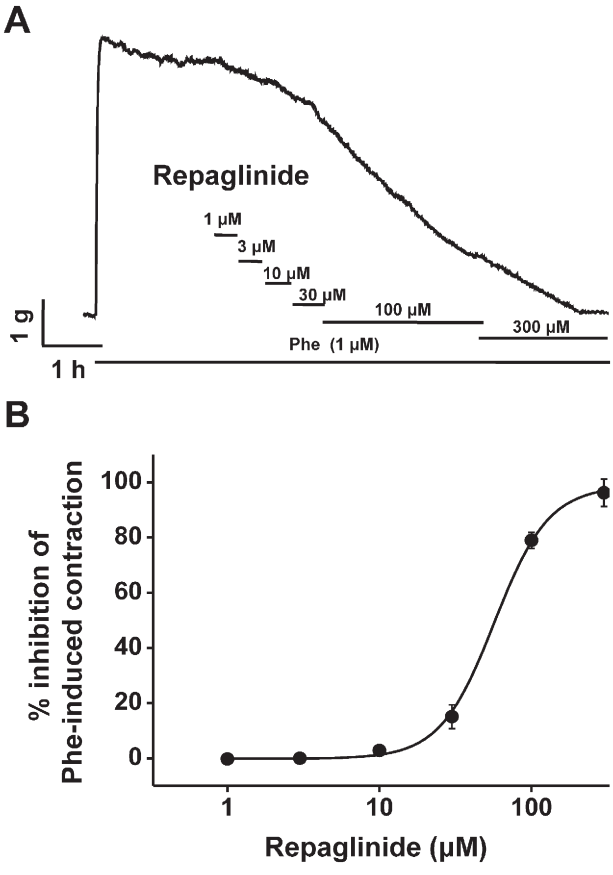

Fig. 1. Vasorelaxant effects of repaglinide on rabbit thoracic aorta rings. (A) Vasorelaxant effect of various concentrations of repaglinide (1, 3, 10, 30, 100, and 300 μM) on Phe-induced pre-contracted aortic rings. (B) Dose-dependent curve of repaglinide-induced vasorelaxation on Phe-induced pre-contracted aortic rings. All $n = 10$.

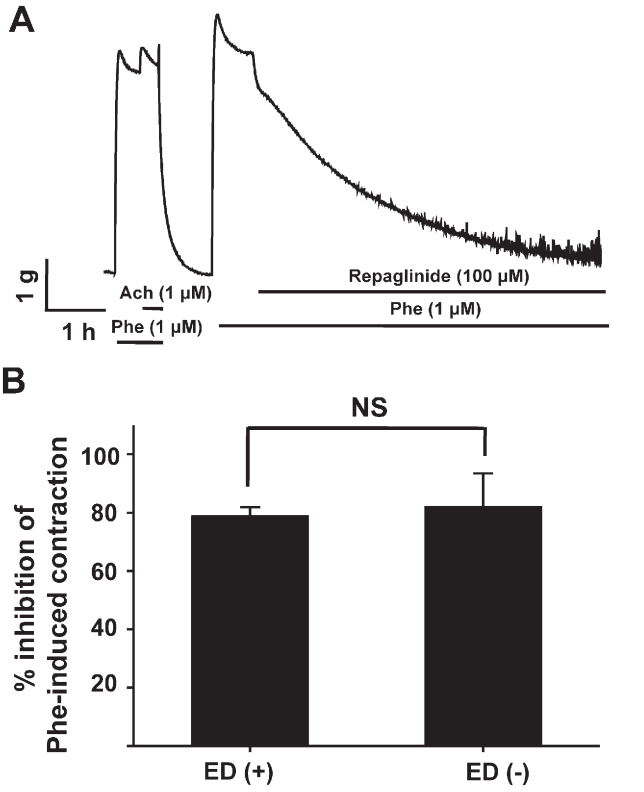

Fig. 2. Vasorelaxant effects of repaglinide on endothelial-intact and -denuded aortic rings. (A) Vasorelaxant effect of repaglinide on endothelial-denuded aortic rings. The endothelium was removed by perfusion of air bubbles and acetylcholine-induced further constriction was regarded as successful removal of the endothelium. (B) Summary of the vasorelaxant effects of repaglinide on endothelial-intact and -denuded aortic rings. $n = 6$. NS = not significant (endothelium-intact vs. endothelium-denuded by Student's *t*-test).

2. Materials and methods

2.1. Vessel preparation and measurement

Male New Zealand White rabbits (2.0–2.5 kg) were anesthetized with heparin (100 U/kg) and sodium pentobarbitone (50 mg/kg) into the ear vein. The procedure was conducted in accordance with the guidelines of the Committee for Animal Experiments of Kangwon National University, South Korea. The thoracic aorta was immediately dissected under a stereomicroscope in normal Tyrode's solution. The isolated thoracic aorta was purified by removal of connective tissue and perivascular adipose in physiological salt solution (PSS), and then cut into ring fragments of 10 mm in length. The rings were fixed to two stainless steel hooks and set in an organ chamber containing oxygenated (95% O_2 and 5% CO_2) PSS at 37 °C for 1 h. The arteries were adjusted to a resting tension of 1 g over a period of 100 min. Endothelial cells were effectively removed by intraluminal perfusion of air bubbles for 5 min. Successful removal of endothelial cells was confirmed by further acetylcholine-induced constriction. Arterial contractility was tested by applying 80 mM high K^+-PSS solution. In high glucose experiments, aortic rings were incubated in PSS containing normal glucose (10 mM) or high glucose (50 mM) for 3 h. Mannitol at 40 mM was added in the normal glucose control to equilibrate osmolarity to that of high glucose.

2.2. Solutions and chemicals

The normal Tyrode's solution containing (mM): 135 NaCl, 5.4 KCl, 0.33 NaH_2PO_4, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 5 HEPES, and 16.6 Glucose, adjusted to pH 7.4 with NaOH. PSS containing (mM): 120 NaCl, 4.7 KCl, 24