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The anti-diabetic drug dapagliflozin induces vasodilation via activation of PKG and Kv channels

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ABSTRACT

Aim: Considering the clinical efficacy of dapagliflozin in patients with type 2 DM and the pathophysiological relevance of Kv channels for vascular reactivity. We investigate the vasodilatory effect of dapagliflozin and related mechanisms using phenylephrine (Phe)-induced contracted aortic rings.

Material and methods: Arterial tone measurement was performed in aortic smooth muscle.

Key findings: Application of dapagliflozin induced vasodilation in a concentration-dependent manner. Pretreatment with the BK_{Ca} channel inhibitor paxilline, the K_{ATP} channel inhibitor glibenclamide, and the Kir channel inhibitor Ba^{2+} did not change dapagliflozin-induced vasodilation. However, application of the Kv channels inhibitor 4-AP effectively inhibited dapagliflozin-induced vasodilation. Application of the Ca^{2+} channel inhibitor nifedipine and the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump inhibitor thapsigargin did not alter the vasodilatory effect of dapagliflozin. Moreover, the adenylyl cyclase inhibitor SQ 22536 and the protein kinase A (PKA) inhibitor KT 5720 had no effect on dapagliflozin-induced vasodilation. Although guanylyl cyclase inhibitors, NS 2028 and ODQ, did not reduce the vasodilatory effect of dapagliflozin, the protein kinase G (PKG) inhibitor KT 5823 effectively inhibited dapagliflozin-induced vasodilation. The vasodilatory effect of dapagliflozin was not affected by elimination of the endothelium. Furthermore, pretreatment with the nitric oxide synthase inhibitor L-NAME or the small-conductance Ca^{2+}-activated K (SKCa) channel inhibitor apamin did not change the vasodilatory effect of dapagliflozin.

Significance: We concluded that dapagliflozin induced vasodilation via the activation of Kv channels and PKG, and was independent of other K^+ channels, Ca^{2+} channels, intracellular Ca^{2+}, and the endothelium.

1. Introduction

Type 2 diabetes mellitus (DM), as a long-term metabolic disorder, is characterized by insulin resistance, increased hepatic glucose output, and defects in insulin secretion by the pancreas [1,2]. Not only is Type 2 DM dangerous, but it is also associated with several complications including ischemic heart disease, vascular dysfunction, nephropathy, and retinopathy [3–6]. For this reason, much effort has been devoted to developing safe and effective anti-diabetic drugs for decades. In fact, several anti-diabetic agents have been developed, such as biguanides, sulfonylureas, meglitinides, alpha-glucosidase inhibitors, thiazolidinediones, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 agonists, and sodium glucose cotransporter 2 (SGLT 2) inhibitors. Among these, SGLT2 inhibitors effectively inhibit glucose-reuptake in renal tubules, which leads to the excretion of glucose in the urine, and consequently reduce blood glucose levels [7,8]. Several types of SGLT2 inhibitors, including dapagliflozin, empagliflozin, and canagliflozin have been developed. Although these drugs effectively reduce blood

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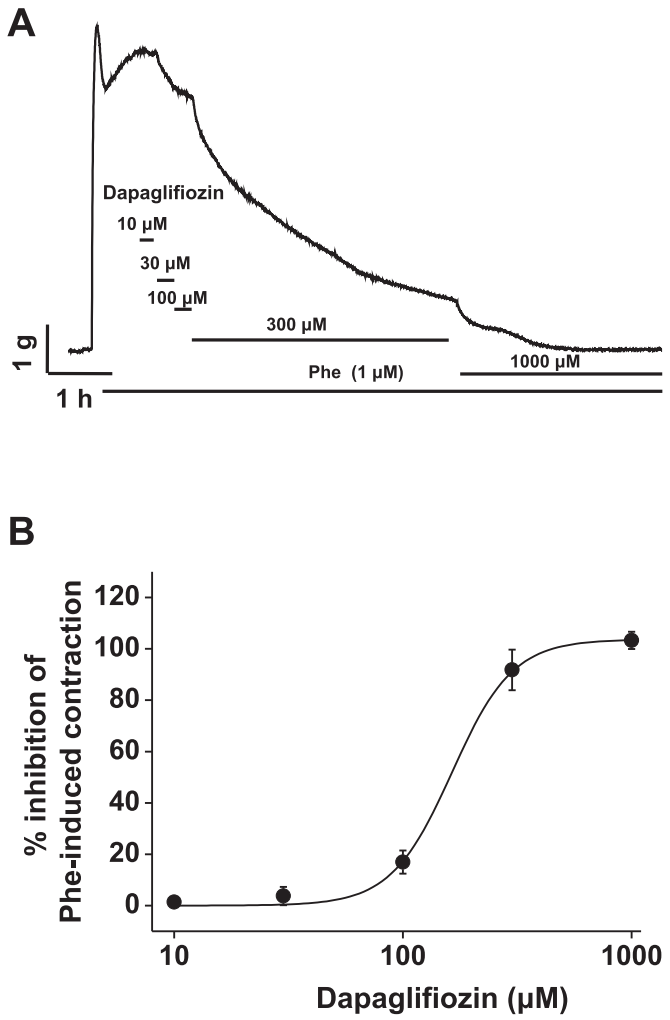

Fig. 1. The effect of dapagliflozin on rabbit aortic rings. (A) Vasodilatory effects of various concentrations (10, 30, 100, 300, and 1000 μM) of dapagliflozin on Phe-induced pre-contracted aortic rings. (B) Dose-dependent curve for the vasodilatory effect of dapagliflozin on Phe-induced pre-contracted aortic rings. All $n = 7$.

glucose level, they also decrease blood pressure [9]. However, the other effects of SGLT2 inhibitors on blood vessels have not been studied. Therefore, the present study investigates the effect of dapagliflozin, a representative SGLT2 inhibitor, on aortic vasoreactivity and related signaling mechanisms.

The resting membrane potential of vascular smooth muscle, which is mainly regulated by K^+ channels, is a key regulator of vascular tone [10,11]. Activation of K^+ channels changes the resting membrane potential to hyperpolarization, thereby relaxing the vasculature [12]. Four types of K^+ channels have been identified in most vascular smooth muscle. Among these, voltage-dependent K^+ (Kv) channels are one of the important channels to regulate resting membrane potential and consequently vascular tone. Indeed, blockade of Kv channels with 4-aminopyridine (4-AP) leads to membrane depolarization and vasoconstriction [10,13]. Kv channels are also closely related to intracellular signaling pathways. Several vasodilators or vasoconstrictors regulate Kv channels through protein kinase A (PKA) and/or protein kinase G (PKG)-related signaling pathways. Therefore, alterations of vascular Kv channels are closely associated with many cellular functions and cardiovascular disease, such as hypertension, diabetes, and hypoxia [14]. Therefore, the effect of some drugs on vascular Kv channels should be investigated to avoid unexpected effects on the vasculature.

Dapagliflozin, an SGLT2 inhibitor, is widely prescribed to treat type 2 DM. Considering the clinical efficacy of dapagliflozin in patients with type 2 DM and the pathophysiological relevance of Kv channels for vascular reactivity, the effect of dapagliflozin on Kv channels and its related cellular signaling pathways should be investigated.

We investigated the vasodilatory effect of dapagliflozin on rabbit thoracic aorta. Dapagliflozin induced vasodilation via activation of Kv channels and PKG, but other K^+ channels, Ca^{2+} channels, and PKA were not involved in dapagliflozin-induced vasodilation. In addition, this effect was completely independent of the endothelium.

2. Materials and methods

2.1. Aorta preparation and tension measurement

All animal experiments were performed under the guidelines for Animal Experiments Committee of Kangwon National University (No. KW-161101-5). Male New Zealand White rabbits (2.2–2.4 kg) were sacrificed with an overdose of heparin (120 U/kg) and sodium pentobarbitone (50 mg/kg). The thoracic aorta was rapidly dissected and the connective tissue and adipose were removed under exposure to normal Tyrode's solution. The cleaned aorta was cut 10 mm in length and the aortic ring was mounted on two parallel hooks in an organ chamber system containing oxygenated (95% O_2 and 5% CO_2) physiological salt solution (PSS). The aortic ring was sustained at a resting tension of 1 g for 100 min at 37 °C. Most experiments were performed using endothelium-intact arteries, except for the experiments in Fig. 6. Endothelium-denuded arteries were prepared by injection of air bubbles into the vessel lumen. High K^+ (80 mM)-induced constriction was used for testing the arterial viability before all experiments.

2.2. Solutions and chemicals

Normal Tyrode's solution for vessel preparation contained (mM): KCl 5.3, NaCl 143, $MgCl_2$ 0.7, $CaCl_2$ 1.7, HEPES 5.2, NaH_2PO_4 0.56, and glucose 12.7. pH was adjusted to 7.4 with NaOH. PSS for tension measurement contained (mM): KCl 5.3, NaCl 123, $CaCl_2$ 1.8, $MgSO_4$ 1.3, $NaHCO_3$ 24, KH_2PO_4 1.4, and glucose 16.8. pH was adjusted to 7.4 with NaOH. 4-aminopyridine (4-AP), phenylephrine (Phe), and $BaCl_2$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in distilled water. Dapagliflozin, glibenclamide, paxilline, SQ 22536, KT 5720, ODQ, KT 5823, nifedipine, thapsigargin, L-NAME, DPO-1, and acetylcholine were purchased from Tocris Cookson (Ellisville, MO) and dissolved in DMSO. Apamin and guangxitoxin were purchased from Tocris Cookson (Ellisville, MO) and dissolved in distilled water.

2.3. Data analysis

Data analysis was performed using Origin v.7.0 software (Microcal Software, Inc., Northampton, MA, USA). The results are expressed as means ± standard error of the mean (S.E.M). Student's *t*-tests were used to evaluate statistical significance. Values of $P < 0.05$ were considered statistically significant.