

Original Article

Endothelium-independent vasorelaxant effect of 20(S)-protopanaxadiol on isolated rat thoracic aorta

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Aim: Ginsenosides are considered to be the major pharmacologically active ginseng constituents, whereas 20(S)-protopanaxadiol [20(S)-PPD] is the active metabolite of ginsenosides in gut. In this study we investigated the effect of 20(S)-PPD on isolated rat thoracic aortas as well as its vasorelaxant mechanisms.

Methods: Aortic rings with or without endothelium were prepared from Wistar rats and suspended in organ-chambers. The changes in tension of the preparations were recorded through isometric transducers connected to a data acquisition system. The aortic rings were precontracted with phenylephrine (PE, 1 $\mu\text{mol/L}$) or high- K^+ (80 mmol/L).

Results: Application of 20(S)-PPD (21.5–108.5 $\mu\text{mol/L}$) caused concentration-dependent vasodilation of endothelium-intact aortic rings precontracted with PE or high- K^+ , which resulted in the EC_{50} values of 90.4 or 46.5 $\mu\text{mol/L}$, respectively. The removal of endothelium had no effect on 20(S)-PPD-induced relaxation. The vasorelaxant effect of 20(S)-PPD was also not influenced by the preincubation with β -adrenergic receptor antagonist propranolol, or with ATP-sensitive K^+ channel blocker glibenclamide, voltage-dependent K^+ channel blocker 4-AP and inward rectifier K^+ channel blocker BaCl_2 , whereas it was significantly attenuated by the preincubation with Ca^{2+} -activated K^+ (BK_{Ca}) channel blocker TEA (1 mmol/L). Furthermore, the inhibition of NO synthesis, cGMP and prostacyclin pathways did not affect the vasorelaxant effect of 20(S)-PPD. In Ca^{2+} -free solution, 20(S)-PPD (108.5 $\mu\text{mol/L}$) markedly decreased the extracellular Ca^{2+} -induced contraction in aortic rings precontracted with PE or high- K^+ and reduced PE-induced transient contraction. Voltage-dependent Ca^{2+} channel antagonist nifedipine inhibited PE-induced contraction; further inhibition was observed after the application of receptor-operated Ca^{2+} channel inhibitor SK&F 96365 or 20(S)-PPD.

Conclusion: 20(S)-PPD induces vasorelaxation via an endothelium-independent pathway. The inhibition of voltage-dependent Ca^{2+} channels and receptor-operated Ca^{2+} channels and the activation of Ca^{2+} -activated K^+ channels are probably involved in the relaxation.

Keywords: Ginseng; 20(S)-protopanaxadiol; vasorelaxation; aortic rings; potassium channels; calcium channels

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Introduction

Arterial hypertension remains a primary global health problem that has a significant effect on cardiovascular morbidity and mortality^[1]. The risk of cardiovascular disease in hypertensive individuals has been well documented^[2], and hypertension may contribute to the reduction of health-related quality of life in patients. It has been estimated that 15% to 20% of

hypertensive patients are not adequately controlled on a dual antihypertensive combination and that three or more different antihypertensive drug classes are required to achieve blood pressure control^[3]. Many synthetic drugs are used to treat hypertension, but they have various adverse effects. Recently, the use of natural herbs has steadily grown because of their low toxicity and well-established therapy strategy^[4]. Many of the plants used in traditional medicine have been investigated for treating cardiovascular disease^[5].

Ginseng (*Panax ginseng* CA Mey) has been widely used in traditional Chinese medicine for over a thousand years to improve health and vigor^[6]. The beneficial effects of ginseng

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have been investigated in disorders of the central nervous systems, cardiovascular system, endocrine system and immune system^[7, 8]. Ginseng also exhibits free radicals scavenging activity and inhibitory effects on human immunodeficiency virus type (HIV) and hepatitis C virus (HCV) proteases^[9, 10]. To date, more than 40 ginsenosides, which are the main bioactive chemical constituents of ginseng, have been reported^[11]. These ginsenosides are classified as protopanaxadiols or protopanaxatriols. The ingested ginsenosides undergo extensive metabolism in the gastrointestinal tract and are deglycosylated into active metabolites by the intestinal microflora^[12, 13]. Previous studies have shown that protopanaxatriol and the ginsenoside Rg3 have endothelium-dependent relaxation effects on isolated rat thoracic aortas by enhancing the release of nitric oxide from endothelial cells^[14, 15]. Other studies have shown that the ginsenoside Rg3 evokes endothelium-independent relaxation in rat aortic rings and that this effect appears to be due to an inhibition of Ca²⁺ influx and stimulation of K⁺ efflux, possibly via the activation of tetraethylammonium (TEA)-sensitive K⁺ channels^[16]. These findings are controversial. However, ginseng contains over twenty ginsenosides, and single ginsenosides have been shown to produce multiple effects in the same tissue^[17]. Ginsenosides exhibit considerable structural variation. These compounds differ from one another in the type and number of sugar moieties, and their site of attachment. The natural glycosides ginsenosides Rb1 and Rg3 are pro-drugs. The 20(S)-ginsenoside Rg3 are transformed into the active compound 20(S)-protopanaxadiol (PPD) by the intestinal microflora, and the active compound is then absorbed and transmitted into the blood^[18]. Orally administered protopanaxadiol-type and protopanaxatriol-type ginsenosides are metabolized to PPD via compound K and 20(S)-protopanaxatriol (PPT) via ginsenoside Rh1, respectively, by gut microbiota^[19]. These metabolites have better bioavailability, owing to their crossing of the intestine-blood and blood-brain barriers^[12]. These compounds exhibit many pharmacological activities similar to those of ginseng. In addition, 20(S)-PPD exhibits anticancer effects in experimental animals and cultured cells. At present, 20(S)-PPD has been developed into a Chinese medicine to assist in radiotherapy and chemotherapy, which is currently being assessed in clinical stage III trials^[20]. Hyun *et al* have shown that 20(S)-PPD has anti-stress effects in immobilized mice^[21]. As an important active ingredient, 20(S)-PPD exhibits numerous pharmacological effects *in vitro* or *in vivo*; however, it remains unknown whether 20(S)-PPD has vasorelaxant effects in rat thoracic aortas.

Therefore, the present study was designed to investigate the vasoactivity of 20(S)-PPD and its possible mechanisms in isolated rat aortic rings with or without endothelium.

Materials and methods

Chemicals and drugs

20(S)-protopanaxadiol was purchased from the National Institute for Food and Drug Control (Beijing, China). Glibenclamide, propranolol, acetylcholine (ACh), barium chloride (BaCl₂), potassium chloride (KCl), phenylephrine hydro-

chloride (PE), tetraethylammonium (TEA), 4-aminopyridine (4-AP), *L*-nitro arginine methyl ester (*L*-NAME), methylene blue (MB), indomethacin, nifedipine and SK&F 96365 were purchased from Sigma Aldrich (St Louis, MO, USA). The other reagents were of analytical purity.

Animals

Three-month-old male Wistar rats (260–280 g) were obtained from the Animal Center of Lanzhou University (Lanzhou, China). The rats were maintained under a 12-h light/dark cycle and had free access to food and water. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the National Institute of Pharmaceutical Education and Research.

Preparation of rat aorta

After anesthetization with chloral hydrate (350 mg/kg), the animals were subsequently decapitated. The chest of each rat was opened, and the descending thoracic aorta was rapidly dissected and placed in 4°C modified Krebs-Henseleit (K-H) solution (mmol/L: NaCl 118.0, KCl 4.7, K₃PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25, CaCl₂ 2.5, *D*-glucose 10.0, pH 7.4). The vessels for the ring segments were carefully excised and cleaned, and the tissues were cut into approximately 3-mm-long segments. For intact tissue preparation, extreme care was taken to avoid endothelial cell damage. When required, the endothelium was removed by gentle rubbing against a paper clip by using a pair of forceps. Aortic rings were suspended in organ chambers containing 10 mL K-H solution at 37°C aerated with 95% O₂ and 5% CO₂. After equilibration under no tension for 15 min, the vessel segments were allowed to equilibrate for 75 min at a resting tension of 2 g. During the equilibration period, the K-H solution was changed every 15 min. Changes in tension were recorded by isometric transducers connected to a data acquisition system (BIOPAC Systems MP150, Goleta, CA, USA). At the beginning of each experiment, rings were exposed for 30 to 45 min to 80 mmol/L KCl, and this process was repeated every 30 to 45 min until the responses were stable (two to three times). The absence of a functional endothelium was verified by the inability of ACh (10 μmol/L) to induce more than 80% relaxation in the aorta rings precontracted with PE (1 μmol/L). Endothelium denudation was considered effectively removed when ACh caused less than 10% relaxation.

The effect of 20(S)-PPD on the contraction induced by PE and KCl in isolated aortic rings

The steady contraction of the endothelium-intact or endothelium-denuded aortic rings was induced by PE (1 μmol/L) or KCl (80 mmol/L). Then, 20(S)-PPD (dissolved in DMSO) was added cumulatively into the K-H solution (21.5, 43.0, 64.5, 86.0, or 108.5 μmol/L) to verify its vasodilation effects. The same volume of DMSO was added to the vehicle control group.

Role of the β-receptor on the effects of 20(S)-PPD

To determine whether the relaxation effects of 20(S)-PPD were

related to the β -receptor, the β -receptor blocker propranolol (1 $\mu\text{mol/L}$) was pre-incubated with endothelium-intact aortic rings for 20 min before PE (1 $\mu\text{mol/L}$) treatment, and 20(S)-PPD was added to the chamber cumulatively.

Role of K^+ channel on the effect of 20(S)-PPD

To investigate the role of K^+ channels in 20(S)-PPD-induced relaxation, endothelium-denuded aortic rings were exposed to the K^+ channel blockers, including 10 mmol/L 4-AP (a predominant inhibitor of voltage-gate K^+ channels, K_v), 1 mmol/L TEA (a blocker of large Ca^{2+} -activated K^+ channels, K_{Ca}), 1 mmol/L BaCl_2 (an inhibitor of the inward rectifier K^+ channels, K_{ir}), and 0.1 mmol/L glibenclamide (an inhibitor of ATP-dependent K^+ channels, K_{ATP}), for 20 min before contraction was induced by PE or KCl. Finally the 20(S)-PPD in cumulative concentrations was added to the chamber and evaluated for 25 min.

Role of 20(S)-PPD on endothelium-intact rings pre-incubated with L-NAME, MB and indomethacin

To investigate the vasorelaxant effect of 20(S)-PPD on the nitric oxide (NO) pathway, NO-cyclic guanosine monophosphate (cGMP) pathway and prostacyclin pathway in endothelium-intact aortic rings, the endothelium-intact aortic rings were pre-incubated with 10 $\mu\text{mol/L}$ NO synthase inhibitor L-NAME, 10 $\mu\text{mol/L}$ cGMP inhibitor MB and 1 $\mu\text{mol/L}$ cyclooxygenase inhibitor indomethacin for 20 min before PE (1 $\mu\text{mol/L}$) pre-contraction.

Role of 20(S)-PPD on extracellular Ca^{2+} -induced contraction

In the first set of experiments, we sought to verify the effect of 20(S)-PPD on the calcium channel. Aortic rings were washed with Ca^{2+} -free K-H solution (containing 50 $\mu\text{mol/L}$ EGTA) four times before PE was added to induce steady contraction. Thereafter, the aortic rings were treated with 20(S)-PPD (108.5 $\mu\text{mol/L}$) for 15 min, and CaCl_2 (in K-H buffer) was added to obtain a concentration-response curve.

In another set of experiments, the depolarization-induced contraction was studied in endothelium-denuded rings stabilized in high- K^+ (contained 80 mmol/L KCl), Ca^{2+} -free K-H solution. The aortic rings were washed for four times with Ca^{2+} -free K-H solution before being incubated with 80 mmol/L KCl until a steady contraction was obtained. Then, the cumulative concentration-response curves of CaCl_2 were recorded in the absence or presence of 20(S)-PPD (108.5 $\mu\text{mol/L}$), which was added 15 min before CaCl_2 treatment.

Role of 20(S)-PPD and SK&F 96365 in PE-induced contraction in the presence of nifedipine

To investigate the effect of 20(S)-PPD on Ca^{2+} influx through ROCCs, we determined the effect of 108.5 mmol/L 20(S)-PPD and the receptor-operated calcium channels (ROCC) blocker SK&F 96365 (50 $\mu\text{mol/L}$) on PE (1 $\mu\text{mol/L}$)-induced contraction in the presence of the voltage-dependent calcium channels (VDCC) blocker nifedipine (10 $\mu\text{mol/L}$). PE was applied twice in the presence of nifedipine, and the aortic rings were treated

with 20(S)-PPD or SK&F 96365 before the second application of PE.

Role of 20(S)-PPD on intracellular Ca^{2+} -induced contraction

To clarify whether intracellular Ca^{2+} release was involved in the aortic relaxation induced by 20(S)-PPD, the rings were exposed to Ca^{2+} -free K-H solution for 15 min before the application of 1 $\mu\text{mol/L}$ PE to induce the first transient contraction (Con1). Then, the rings were washed three times with normal K-H solution and incubated for at least 40 min to refill the intracellular Ca^{2+} stores. Subsequently, the medium was rapidly replaced with Ca^{2+} -free solution, and the rings were incubated for an additional 15 min. The second contraction (Con2) was then induced with 1 $\mu\text{mol/L}$ PE in the absence or presence of 20(S)-PPD (108.5 $\mu\text{mol/L}$) before the application of PE. The ratio of the second contraction to the first contraction (Con2/Con1) was calculated.

Statistical analysis

All data are expressed as the mean \pm SEM. Student's *t*-test was used to compare the data. Curves were compared using one-way ANOVA followed by Tukey's test. *P*-values less than 0.05 were considered to be statistically significant. EC_{50} was analyzed by using OriginPro 8.0 with the DoseResp Fittin.

Results

20(S)-PPD induced vasodilation in PE- or KCl-precontracted aortic rings

In the absence of any vasoactive agent, 20(S)-PPD (21.5, 43.0, 64.5, 86.0, and 108.5 $\mu\text{mol/L}$) showed no obvious effects on the basal tension of aortic rings, as shown in our previous study, whereas it resulted in a concentration-dependent relaxation in the endothelium-intact and denuded aortic rings pre-contracted with 1 $\mu\text{mol/L}$ PE (EC_{50} =90.4, 91.7 $\mu\text{mol/L}$) or 80 mmol/L KCl (EC_{50} =46.5, 43.2 $\mu\text{mol/L}$) (Figure 1A and 1B).

Effect of propranolol on 20(S)-PPD induced relaxation

Pre-incubation with propranolol (1 $\mu\text{mol/L}$), an inhibitor of β -receptor, did not modify the vasorelaxant effect of 20(S)-PPD (EC_{50} = 83.8, 81.6 $\mu\text{mol/L}$) (Figure 2), thus suggesting that the vasodilation effect of 20(S)-PPD was not mediated by the β -receptor.

Role of K^+ channel in 20(S)-PPD-induced relaxation

After 30 min of pre-incubation with different K^+ channel blockers, including 4-AP (0.1 mmol/L), BaCl_2 (1.0 mmol/L), glibenclamide (0.1 mmol/L) and TEA (1 mmol/L), the aortic rings were contracted with PE or KCl and then exposed to the cumulative concentrations of 20(S)-PPD. Here, 4-AP, BaCl_2 and glibenclamide exhibited no obvious effects on the vasodilation effect of 20(S)-PPD. However, TEA (1 mmol/L) pre-treatment attenuated 20(S)-PPD-induced relaxation (Figure 3A and 3B). 20(S)-PPD exhibited different EC_{50} values with different blockers. The EC_{50} values in Figure 3A were 83.1, 104.9, 80.9, 82.1, and 81.3 $\mu\text{mol/L}$ in control, TEA, 4-AP, BaCl_2 , and glibenclamide group, respectively. In Figure 3B, the EC_{50}

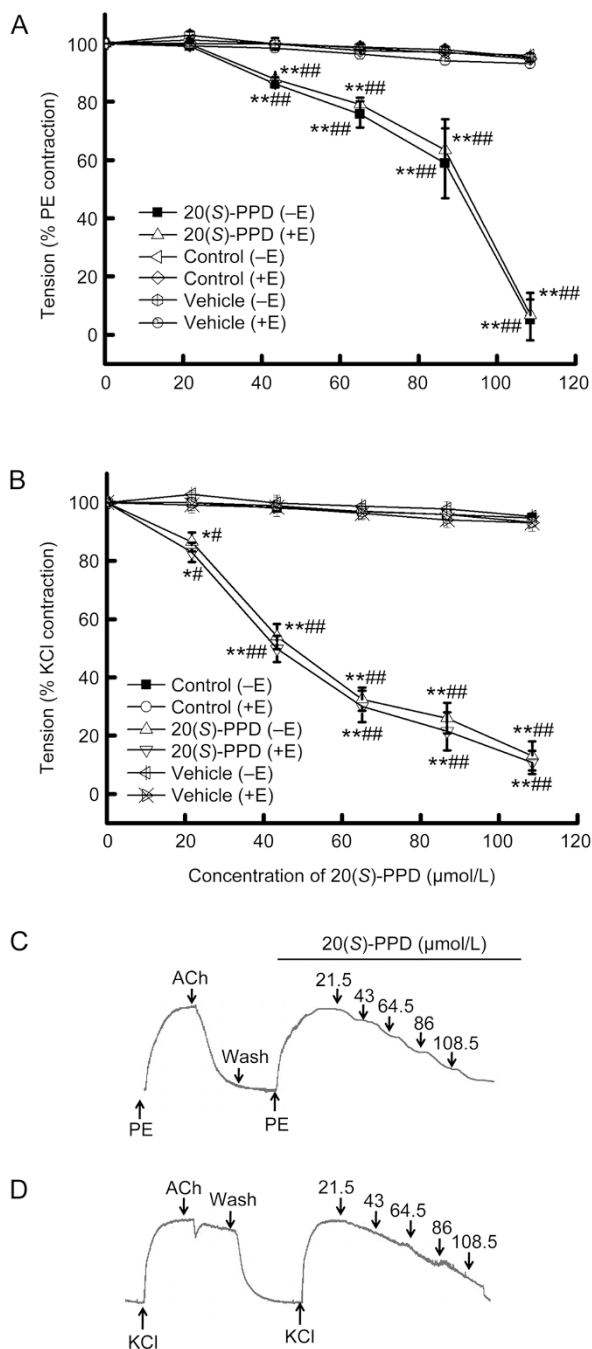


Figure 1. Effect of 20(S)-PPD on tension in PE ($1 \mu\text{mol/L}$, A) or KCl (80 mmol/L , B) pre-contracted aortic rings with endothelium (+E, $n=8$) or without endothelium (-E, $n=8$). The traces of 20(S)-PPD induced-relaxation in endothelium-intact aortic rings pre-contracted by PE (C) or KCl (D). Tension (%) indicates the percentage of PE or KCl-induced contraction. Values are expressed as the mean \pm SEM. * $P<0.05$, ** $P<0.01$ vs the control group. # $P<0.05$, ## $P<0.01$ vs vehicle control group. In the vehicle control, an equivalent volume of DMSO was used instead of 20(S)-PPD.

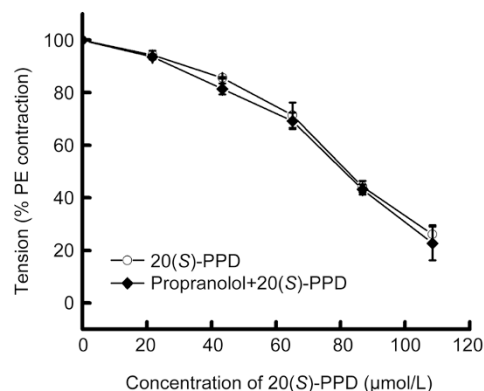


Figure 2. Effect of propranolol ($1 \mu\text{mol/L}$) on 20(S)-PPD-induced relaxation in PE pre-contracted endothelium-intact aortic rings. Tension (%) indicates the percentage of PE-induced contraction. Values are expressed as the mean \pm SEM. $n=8$.

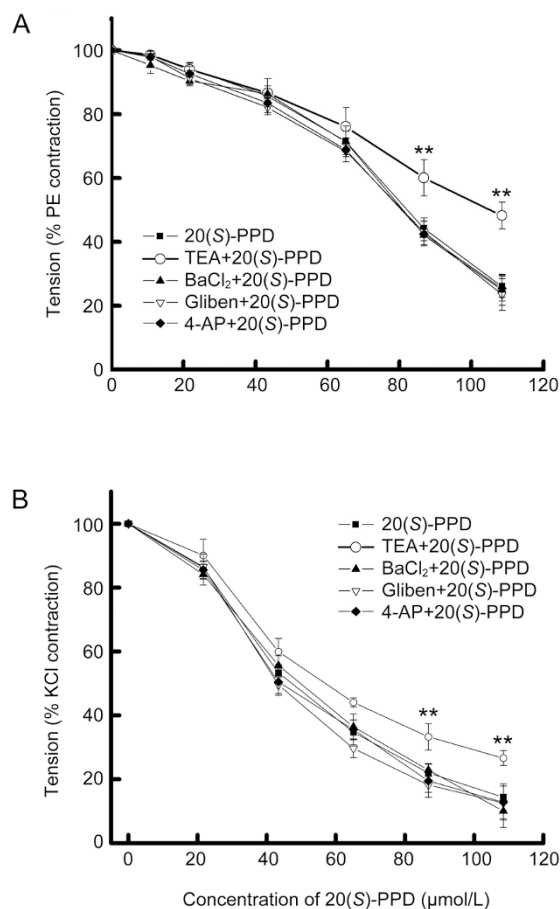


Figure 3. Effect of 4-AP (0.1 mmol/L), BaCl₂ (1 mmol/L), glibenclamide (Gliben 0.1 mmol/L), TEA (1 mmol/L) on 20(S)-PPD induced relaxation in aortic rings pre-contracted with PE ($1 \mu\text{mol/L}$) (A) or KCl (80 mmol/L) (B). Tension (%) indicates the percentage of PE or KCl-induced contraction. Values are expressed as the mean \pm SEM. $n=8$. * $P<0.05$ vs 20(S)-PPD group.

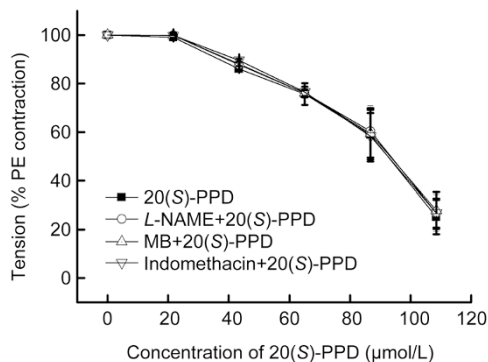


Figure 4. The relaxant effect of 20(S)-PPD on PE (1 $\mu\text{mol/L}$)-pre-contracted aortic rings in the presence or absence (control) of L-NAME (10 $\mu\text{mol/L}$), MB (10 $\mu\text{mol/L}$) and indomethacin (1 $\mu\text{mol/L}$). Values are expressed as the mean \pm SEM. $n=8$.

values were 45.9, 55.1, 48.5, 43.1, and 44.3 $\mu\text{mol/L}$ in control, TEA, 4-AP, BaCl₂, and glibenclamide group.

Effect of 20(S)-PPD on endothelium-intact rings pre-incubated with L-NAME, MB, and indomethacin

We examined the vasorelaxant effect of 20(S)-PPD (21.5, 43.0, 64.5, 86.0, and 108.5 $\mu\text{mol/L}$) on the NO synthesis, cGMP and prostacyclin pathways. The aortic rings were pre-incubated with different inhibitors. As shown in Figure 4, L-NAME, MB and indomethacin did not alter the relaxation induced by 20(S)-PPD.

Effect of 20(S)-PPD on extracellular Ca²⁺-induced contraction and Ca²⁺ channels

We examined the vasorelaxant effect of 20(S)-PPD (108.5 $\mu\text{mol/L}$) on extracellular Ca²⁺-induced contractions via ROCCs and VDCCs. PE activated ROCC and regulated the influx of extracellular Ca²⁺, which caused stable contraction. Therefore, the effect of 20(S)-PPD on ROCC was investigated in Ca²⁺-free K-H solution. The cumulative addition of Ca²⁺ in a Ca²⁺-free K-H solution containing PE or KCl induced a concentration-dependent contraction in the aortic rings. Pre-incubation of the rings with 20(S)-PPD (108.5 $\mu\text{mol/L}$) significantly inhibited the Ca²⁺-induced contraction stimulated with both PE (Figure 5A) and KCl (Figure 5B).

Effect of 20(S)-PPD and SK&F 96365 on PE-induced contraction in the presence of nifedipine

As shown in Figure 6, nifedipine inhibited PE-induced contraction. Further inhibition was observed after the application of SK&F 96365 or 108.5 $\mu\text{mol/L}$ 20(S)-PPD. A combination of SK&F 96365 and nifedipine further decreased the PE-induced contractions. As a result, nifedipine blocked VDCCs at first, and SK&F 96365 blocked ROCCs in sequence. Similarly, 108.5 $\mu\text{mol/L}$ 20(S)-PPD decreased PE-induced contractions in the presence of nifedipine, thus suggesting that 20(S)-PPD inhibits the entry of extracellular Ca²⁺ via ROCCs activated by PE.

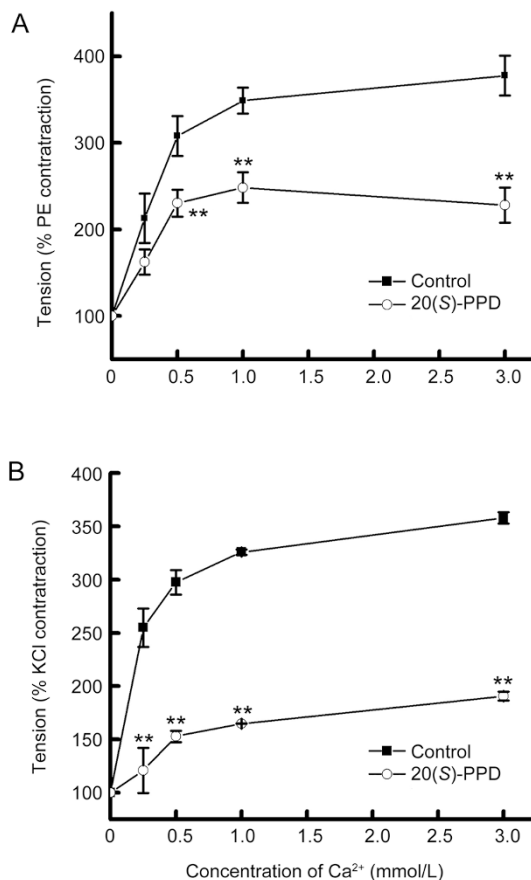


Figure 5. Effect of 20(S)-PPD (108.5 $\mu\text{mol/L}$) on the cumulative-contraction induced by influx of extracellular Ca²⁺ in endothelium-denuded aortic rings pre-contracted with PE (1 $\mu\text{mol/L}$) (A) or KCl (80 mmol/L) (B) in Ca²⁺ free solution. Tension (%) indicates the percentage of PE or KCl induced contraction before Ca²⁺ addition. Values are expressed as the mean \pm SEM. $n=8$. ** $P<0.01$ vs the control group.

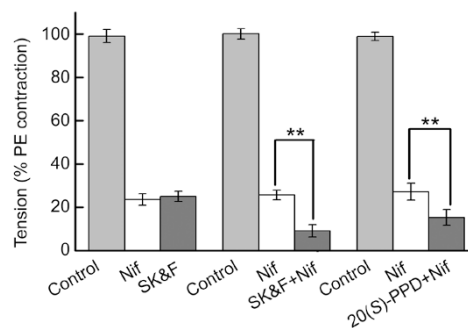


Figure 6. The effects of 20(S)-PPD (108.5 $\mu\text{mol/L}$) and SK&F 96365 (SK&F 50 $\mu\text{mol/L}$) in the presence of nifedipine (Nif 10 $\mu\text{mol/L}$) on PE-induced contraction in endothelium-denuded aortic rings. Values are expressed as the mean \pm SEM. $n=8$. ** $P<0.01$.

Effect of 20(S)-PPD on intracellular Ca²⁺ release

PE also induced transient contraction because of the release

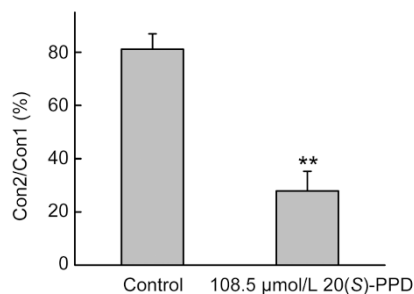


Figure 7. Effect of 20(S)-PPD (108.5 μmol/L) on PE-induced transient contraction in endothelium-denuded aortic rings. Con2/Con1 (%) refers to the ratio of the second contraction to the first contraction. Values are expressed as the mean±SEM. $n=8$. ** $P<0.01$ vs the control group.

of intracellular Ca^{2+} from sarcoplasmic reticulum (SR) in Ca^{2+} -free solution. A second contraction was then induced by PE (1 μmol/L) in the absence or presence of 20(S)-PPD (108.5 μmol/L). As shown in Figure 7, pre-incubation with 20(S)-PPD (108.5 μmol/L) markedly decreased the PE-induced transient contraction ratio (con2/con1).

Discussion

In hypertension, vasoreactivity is a major factor for the treatment of hypertension because it affects circulation and blood pressure in the cardiovascular system. Moreover, vasoreactivity is a basic, important phenomenon that directly influences arteries of the circulatory system. Therefore, many researchers have investigated the vasorelaxant effects of various plant extracts as hypotensive agents by using aortic rings^[22–24]. The endothelium plays key roles in the maintenance of vascular architecture and function, including vascular tone, by regulating the underlying smooth muscle layer through the release of both endothelium-derived relaxing factors, such as nitric oxide (NO), prostaglandins, and endothelium-derived hyperpolarizing factor (EDHF), and vasoconstriction factors^[25]. Ginseng has been used in the Asian pharmacopeia as a traditional medicinal plant for treating illness, restoring homeostasis, promoting longevity and, in particular, for controlling cardiovascular disease^[26]. Han et al have reported that the oral administration of red ginseng decreases the systolic blood pressure, whereas the authors observed no obvious effect on diastolic blood pressure in essential hypertensive patients^[27]. The active components and the underlying mechanisms of ginseng remain unknown. This study provides evidence that 20(S)-PPD decreases tension in both endothelium-intact and endothelium-denuded aortic rings. In our experiment, the functional removal of endothelium did not significantly attenuate the 20(S)-PPD-induced relaxation in aortic rings pre-contracted with PE or KCl, thus suggesting that the vasorelaxant effect of 20(S)-PPD was endothelium-independent.

Several possible mechanisms may be involved in the endothelium-independent vasorelaxant effect of 20(S)-PPD, such as the activation of β-adrenergic receptors, opening of K^+ channels, blockade of extracellular Ca^{2+} influx, and inhibition of the

release of Ca^{2+} from the sarcoplasmic reticulum.

Specific activation of α1 receptors induces vasoconstriction and increases blood pressure. In contrast, vascular smooth muscle relaxes after β-adrenergic receptor stimulation^[28–30]. In this study, propranolol, an inhibitor of the β-adrenergic receptor, did not attenuate 20(S)-PPD-induced relaxation. Therefore, the effect of β-adrenergic-mediated vasorelaxation was not involved in the pathway.

K^+ channels play an important role in regulating resting arterial membrane potential and vascular tone^[31–33]. The direct activation of K^+ channels on arterial smooth muscle cells generally hyperpolarizes the cell membrane, results in the inhibition of Ca^{2+} influx through VDCC, and interrupts smooth muscle contraction. To date, several distinct types of K^+ channels have been identified in vascular smooth muscle: voltage-dependent K^+ (K_v) channels, Ca^{2+} -activated K^+ (BK_{Ca}) channels, ATP-sensitive K^+ (K_{ATP}) channels, and inward rectifier K^+ (Kir) channels. These channels are blocked by 4-AP, TEA, glibenclamide and BaCl_2 , respectively^[31, 33, 34]. This study showed that 20(S)-PPD-induced relaxation was attenuated by TEA, a blocker of BK_{Ca} , but not 4-AP, glibenclamide and BaCl_2 , thus indicating that BK_{Ca} channel activation partially contributed to 20(S)-PPD-induced relaxation in endothelium-denuded arteries. Moreover, large conductance BK_{Ca} contributed to the negative feedback regulation of vascular smooth muscle tone, and the activation of BK_{Ca} can limit the smooth muscle contraction elicited by vasoconstrictors^[31].

Two major types of transmembrane Ca^{2+} channels have been reported in vascular smooth muscle: ROCC and VDCC. These channels are activated in the presence of PE or high extracellular K^+ ^[35, 36]. PE activates the ROCC and regulates the influx of extracellular Ca^{2+} , which in turn causes the aorta to contract topically^[37]. Moreover, PE activates the specific IP_3 receptor channel in sarcoplasmic reticulum membranes and induces release of Ca^{2+} from the SR, thus causing transient vascular smooth muscle contraction^[38, 39]. In our study, the PE-induced transient contraction of aortic rings was inhibited by 20(S)-PPD (Figure 7), a result suggesting that 20(S)-PPD inhibits vasoconstriction induced by the IP_3 receptor by regulating the release of Ca^{2+} from the SR. Moreover, PE induced transient and tonic vasoconstriction in Ca^{2+} -free K-H solution. Aortic rings were stimulated with PE in Ca^{2+} -free K-H solution to obtain steady contraction, and accumulation of Ca^{2+} induced the influx of extracellular Ca^{2+} through the ROCC, which caused tonic contraction. 20(S)-PPD significantly inhibited the contraction of aortic rings induced by Ca^{2+} in PE pre-contracted aortic rings. Otherwise, the aortic rings were contracted by high K^+ , thereby inducing the depolarization and Ca^{2+} influx through VDCC^[40]. Our results showed that 20(S)-PPD also markedly inhibited Ca^{2+} -induced vasoconstriction in high K^+ solution. This finding indicated that 20(S)-PPD inhibits the vasoconstriction induced by the influx of extracellular Ca^{2+} through the ROCC and VDCC. Ginseng modulates blood pressure, metabolism and immune functions, and the mechanism of action for ginseng had not been known until ginsenosides were isolated. 20(S)-PPD is one type of ginsen-

osides, and more attention should be paid to its pharmacological effects on cardiovascular diseases.

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Author contribution

Experiments were conceived and designed by Lu GAN, Hong ZHANG, and Zhen-hua WANG; Experiments were performed by Lu GAN and Hui ZHOU; Data were analyzed by Lu GAN, Xin ZHOU, Chao SUN, Jing SI, and Rong ZHOU. The paper was written by Lu GAN and Zhen-hua WANG. Ji LI and Cheng-jun MA helped to polish the language of the manuscript.

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