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Vasodilatory Effects of Aloperine in Rat Aorta and Its Possible Mechanisms

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Abstract

The aim of this study was to investigate the vasodilatory effects of aloperine, one of main alkaloid was extracted from *Sophora alopecuroides*, on rat isolated thoracic aortic rings and its possible mechanisms. The isolated aortic arteries from normotensive Sprague Dawley rats were pre-contracted with phenylephrine (1×10^{-6} M) or KCl (60 mM). Then, aloperine ($3.44 \times 10^{-3} – 17.21 \times 10^{-3}$ M) was added cumulatively and the tension curves was observed and recorded. The changes in tension in both endothelium-intact and endothelium-denuded aortic rings were also recorded. Afterwards, the interaction between aloperine with NG-nitro-L-arginine methylester (0.1 mM), indomethacin (1×10^{-3} mM), tetraethylammonium (10 mM), 4-aminopyridine (5 mM), $BaCl_2$ (1 mM) and glibenclamide (0.01 mM) was evaluated. In this study, aloperine caused concentration-dependent relaxations in aortic rings precontracted with phenylephrine, but this effect was not observed in KCl-pretreated rings. Removal of endothelium showed no influence on vasodilatory effects of aloperine. In addition, preincubation with NG-nitro-L-arginine methylester and indomethacin did not inhibit the vasodilatory effects of aloperine, suggesting that the vasodilative action is endothelium-independent. Relaxant responses to aloperine were inhibited by tetraethylammonium and 4-aminopyridine. However, the vasorelaxant effect of aloperine was also not influenced by the preincubation with $BaCl_2$ and glibenclamide. These findings suggest that aloperine-induced vasorelaxation effects are mainly due to the operations of voltage-operated potassium channels and ATP-sensitive potassium channels.

Key Words: aloperine, ATP-sensitive potassium channels, endothelium independent, vasodilation, voltage-operated potassium channels

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Introduction

Cardiovascular disease, including peripheral arterial hypertension and pulmonary hypertension etc., is a kind of common and frequent disease, which is a serious threat to life and health of mankind (12, 30). With the increased understanding of the pathogenesis of cardiovascular disease, accumulating evidence indicates that vasodilatation plays an important role in treatment of the disease (4, 15, 40). So, drugs with the properties of vascular relaxation have become the focus of the pharmaceutical development.

Over the past decades, traditional Chinese medicine received more and more attention because of its advantages, such as multi-target efficacy, rich in resources, fewer side effects, low cost (42). *Sophora alopecuroides* is a widely used traditional Chinese herbal medicine which commonly used as antibacterial, antipyretic, anti-inflammatory and analgesic (13, 22, 42, 43). Years of pharmacological and preclinical research proved that *sophora alopecuroides* has a protective effect on the cardiovascular system, such as anti-arrhythmia and protecting myocardial ischemia (14). Aside from that, matrine, an alkaloid extracted from *sophora alopecuroides*, could antagonize phenylephrine-induced contraction of aortic rings in guinea pigs (45). In addition, previous studies have shown that aloperine (Fig. 1), which is another major alkaloid of *sophora alopecuroides*, has protective effects on ischemia-reperfusion induced renal injury (16). These phenomena aroused our interest in the vasorelaxation effects of aloperine.

Concerning the aforementioned, the present study was designed to investigate the vasodilation abilities of aloperine and then to elucidate its possible mechanisms in isolated rat aortic rings.

Materials and Methods

Experiment Animals and Equipment

Sprague Dawley rats weighing 250-300 g were provided by experimental Animal Center of Ningxia Medical University (certificate no. SYXK Ningxia 20160001). Experiment animals were housed under standard temperature (22 ± 2°C), humidity (35%) and light conditions (12 h light–dark cycles) with free access to food and water. The experiment was performed after the rats were fed adaptively for 1 week. All experiments were reviewed and approved by the Animal Experimental Committee Ningxia Medical University.

Chemicals and Drugs

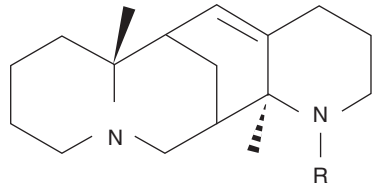

Fig. 1. The chemical structure of aloperine.

The following compound, drugs and chemicals were used in this study: Aloperine (purity > 98.0%) (Dushun biological chemical company, Ningxia, China); NG-nitro-L-arginine methylester (L-NAME) and glibenclamide (Jianglai Chemical company, Jiangsu, China); phenylephrine hydrochloride hydrochloride (PE) (Hefeng Pharmaceutical Company, Shanghai, China), acetylcholine (ACh), 4-aminopyridine (4-AP), tetraethylammonium (TEA) and barium chloride ($BaCl_2$) (Sigma Chemical Company, MO, USA); indomethacin (Aladdin Reagent Company, Shanghai, China); potassium chloride (KCl) (Mingshen Pharmaceutical Company, Zhejiang, China); sodium hydrogen carbonate ($NaHCO_2$), glucose, calcium chloride hexahydrate ($CaCl_2{\cdot}2H_2O$), magnessium sulfate anhydrous ($MgSO_4{\cdot}7H_2O$), sodium chloride (NaCl) (Damao Chemical Reagent Factory, Tianjing, China).

Isolated rat aorta was maintained in Krebs salt solution (118 mM NaCl; 4.7 mM KCl; 25 mM $NaHCO_3$; 2.5 mM $CaCl_2{\cdot}2H_2O$; 1.2 mM KH_2PO_4; $MgSO_4{\cdot}7H_2O$ 1.2; 10 mM glucose; pH 7.4, temperature 37°C) with the mixed standard gas of 95% O_2 + 50% CO_2.

In this experiments, aloperine is dissolved by Krebs salt solution: Firstly, 80 mg of aloperine plus 10 ml Krebs salt solution r to prepare a mother liquor with a concentration of 3.44×10^{-2} M, and then diluted as needed.

Preparation of Isolated Rat Thoracic Aortic Rings

The thoracic aorta was rapidly removed and placed in 4°C Krebs salt solution after the rats were anesthetized with urethane (100 mg/kg). Subsequently, isolated rat thoracic aorta was carefully excised and cleaned and then cut transversely into rings about 3-5 um. The rings were suspended in a 20 ml organ bath chamber containing Krebs salt solution which preheated at 37°C and were mounted in a BL-420s Biological Functional System (Chengdu Technology & Market Company, Sichuan, China) with two steel wires, one fixed and another one connected to tension transducer. Afterwards, aortic rings were stretched with a resting tension of 2 g and were allowed at least 1 h for equilibration be-

fore testing. The thoracic aortic rings were exposed twice to 30 mM KCl. If the difference between two maximum contraction amplitudes is less than 5%, the ring is considered to functional integrity. The endothelium integrity was assessed by adding 1.0 μM ACh after the ring was pre-contracted with PE for 15 min. When ACh induced relaxation reached to 60-90%, the endothelium was regarded as intact, otherwise endothelium is not complete.

Vasodilatory Effects of Aloperine in Rat Thoracic Aortic Rings

To evaluate whether aloperine affects normal aortic rings, the rat thoracic aortic rings without pretreatment were exposed to this alkaloid (3.4429 – 17.2147 M), which was added cumulatively.

Once the sustained contraction was achieved by PE (1×10^{-6} M), increasing concentrations of aloperine ($3.44 - 17.21 \times 10^{-2}$ M) were added cumulatively into the Krebs salt solution. Besides, the same volume of vehicle as that used in the administration with aloperine was added to the control group. Afterwards, the vasorelaxation response of endothelium-intact rings was observed.

Vasodilatory Effects of Aloperine in Endothelium-Denuded Rat Thoracic Aorta Rings

In order to investigate the role of the endothelium in vasorelaxation to aloperine, the endothelium was removed using forceps to lightly rub the inner surface of the rings. The thoracic aorta rings without endothelium integrity were contracted steadily using PE (1×10^{-6} M). Then, aloperine ($3.44 \times 10^{-3} - 17.21 \times 10^{-3}$ M) or the same volume of vehicle was added cumulatively and the tension curves was observed and recorded.

Effects of L-NAME and Indomethacin on Vasorelaxation of Aloperine in Rat Thoracic Aortic Rings

In the experimental and control group, before the application of PE (1×10^{-6} M), the aortic rings were exposed respectively to L-NAME (0.1 mM) and indomethacin (1×10^{-3} mM) for 20 min. After the stable vasoconstriction was achieved, aloperine ($3.44 \times 10^{-3} - 17.21 \times 10^{-3}$ M) was added cumulatively. The vasorelaxation effects were calculated as the percentages of relaxation as compared between the data obtained in the control group and the aloperine treated group.

Role of Potassium Channel in Vasorelaxation Effect of Aloperine in Endothelium-Denuded Rat Thoracic Aorta Rings

To evaluate the role of potassium (K^+) channel in vasorelaxation effect of aloperine, the rat thoracic aorta rings were incubated respectively with 10 mM TEA (blocker of different subtypes of Ca^{2+}-activated potassium channels), 5 mM 4-AP (blocker of many classes of voltage-operated potassium channels), 1 mM $BaCl_2$ (blocker of inward rectifier potassium channels) and 0.01 mM glibenclamide (blocker of ATP-sensitive potassium channels) for 20 min after pro-contraction of PE (1×10^{-6} M). When the constriction was stabilized, aloperine ($3.44 \times 10^{-3} - 17.21 \times 10^{-3}$ M) was added in Krebs salt solution cumulatively.

Statistical Analysis

The results were presented as the mean and SEM, and analyzed with SPSS 17.0. Contraction (%) indicates the percentage of PE or KCl induced contraction in or without administration of aloperine. An independent sample *t*-test was used to compare data between two groups, and one-way analysis of variance following by *Post hoc* Dunnett's test was used for multiple comparisons. For all the statistical tests, $P < 0.05$ was considered statistical significant.

Results

The Rffects of Aloperine on Normal Rat Thoracic Aortic Rings

The present study shown that the data of tension that obtained from aortic rings was no obvious difference in the presence and absence of aloperine (Fig. 2), indicating that aloperine did not affect normal aortic rings.

The Effects of Aloperine on the Contraction Induced by PE and KCl in Rat Thoracic Aortic Rings

Compared with the control group, aloperine caused concentration-dependent vasorelaxant effect on PE-induced contraction in thoracic aorta rings ($P < 0.05$ and $P < 0.01$, Fig. 3). Whereas, treatment with aloperine show no obvious change in tension in pre-contracted aortic rings induced by KCl (Fig. 3). These results illustrate that aloperine cannot relax the KCl pre-contracted rings.

The Effect of Aloperine on the Contraction Induced by PE in Endothelium-Denuded Rat Thoracic Aortic Rings

In this study, there are no differences in the contraction induced by PE between endothelium-intact and endothelium-denuded thoracic aortic

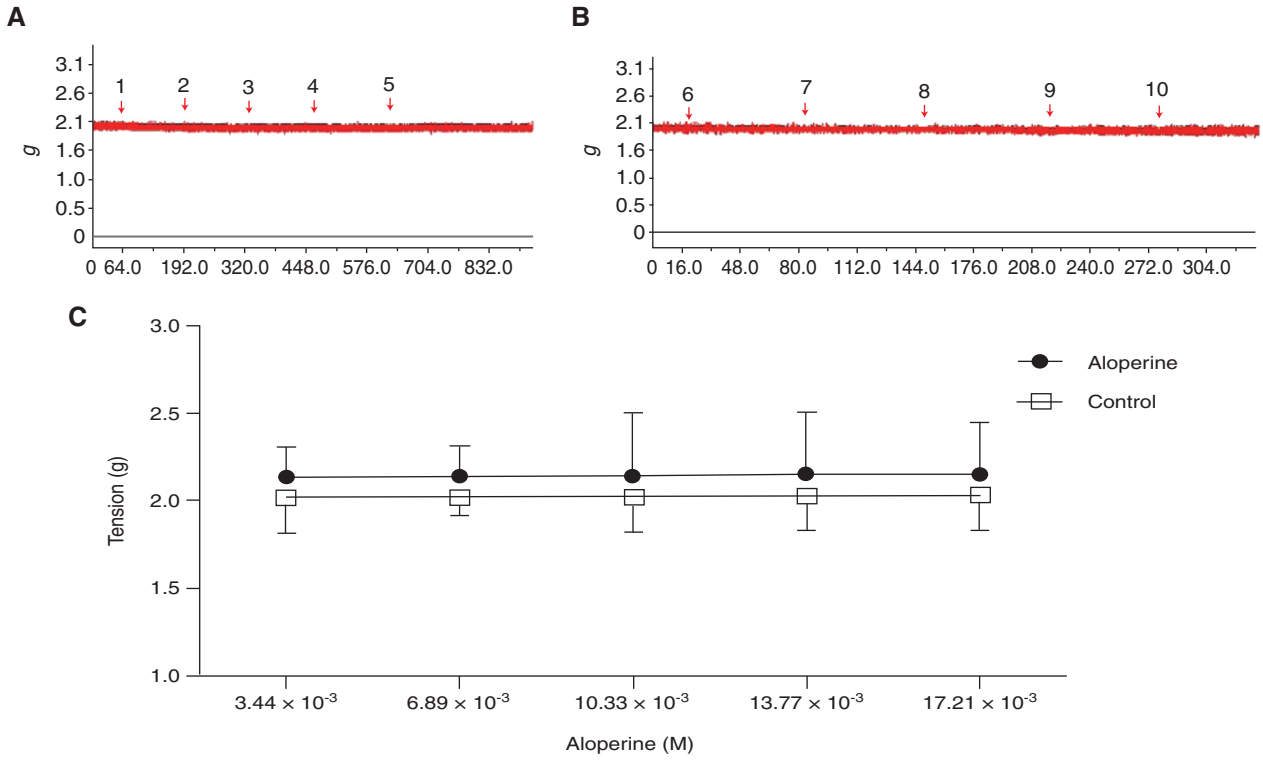

Fig. 2. Influences of different concentrations of aloperine on untreated rat thoracic aortic rings. (A) The typical original trace of aloperine group: (1-5) Adding aloperine $3.44 \times 10^{-3} – 17.21 \times 10^{-3}$ M cumulatively; (B) The typical original trace of control group: (6-10) Adding same dose of solvent; (C) The value of tension of aloperine on untreated rat thoracic aortic rings (n = 6 per group). Data are expressed as the mean ± SEM.

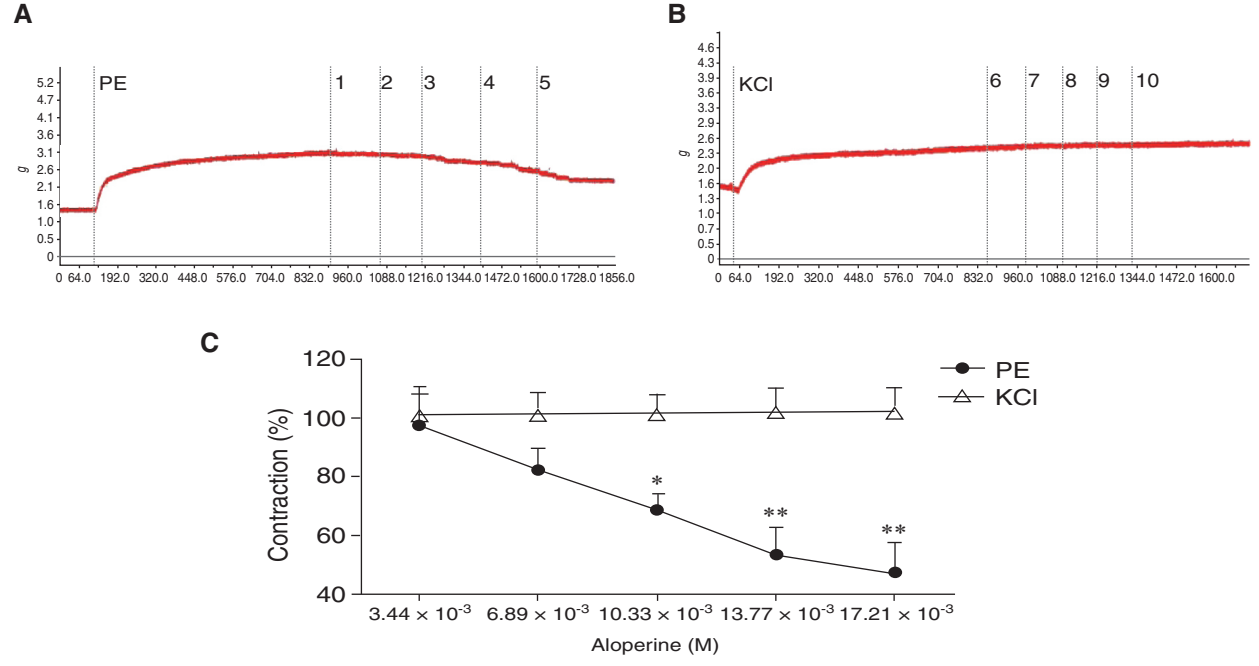

Fig. 3. Respective influences of aloperine on PE and KCl pre-contracted rat thoracic aortic rings. (A) The typical original trace of PE pre-contracted group: (1-5) Adding aloperine $3.44 \times 10^{-3} – 17.21 \times 10^{-3}$ M cumulatively; (B) The typical original trace of KCl pre-contracted group: (6-10) Adding aloperine $3.44 \times 10^{-3} – 17.21 \times 10^{-3}$ M cumulatively; (C) The value of tension of aloperine on PE and KCl pre-contracted rat thoracic aortic rings (n = 6 per group). Data are expressed as the mean ± SEM.

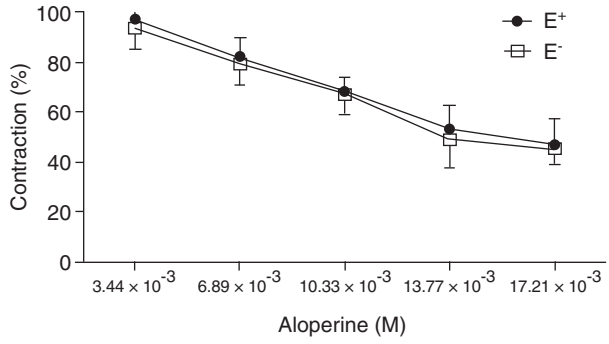

Fig. 4. Respective influences of aloperine on rat endothelium-intact (E^+) and endothelium-denuded (E^-) thoracic aortic rings (n = 6 per group). Data are expressed as the mean ± SEM.

rings (Fig. 4). It is worth noting that the percentage of contraction of aloperine in between endothelium-denuded isolated aortic rings and endothelium intact rings were not significantly different (Fig. 4).

Effects of L-NAME and Indomethacin on Vasorelaxation of Aloperine in Rat Thoracic Aortic Rings

In current study, after pretreatment with L-NAME, a NOS inhibitor, vasorelaxation of aloperine in rat thoracic aortic rings was not reduced (Fig. 5). The vasorelaxant effects of aloperine remained similarly unchanged in endothelium intact rings that were contracted by PE and incubated for 20 min with indomethacin which is a COX inhibitor ($P < 0.05$ and $P < 0.01$, Fig. 5).

Role of K^+ Channel in Vasorelaxation Effects of Aloperine in Rat Thoracic Aortic Rings

In aortic rings, neither Ca^{2+}-activated potassium (K_{ca}) channels inhibitor TEA nor inward rectifier potassium (K_{ir}) channels inhibitor $BaCl_2$ affected vasorelaxation of aloperine ($P < 0.05$ and $P < 0.01$, respectively, Fig. 6). However, pretreatment with voltage-operated potassium (K_v) channels inhibitor 4-AP and ATP-sensitive potassium (K_{ATP}) channels inhibitor glibenclamide significantly respectively antagonized the vasorelaxation effect of aloperine ($P < 0.05$ and $P < 0.01$, respectively, Fig. 6), suggesting that K_{ir} channels is seem to be involved in such vascular action.

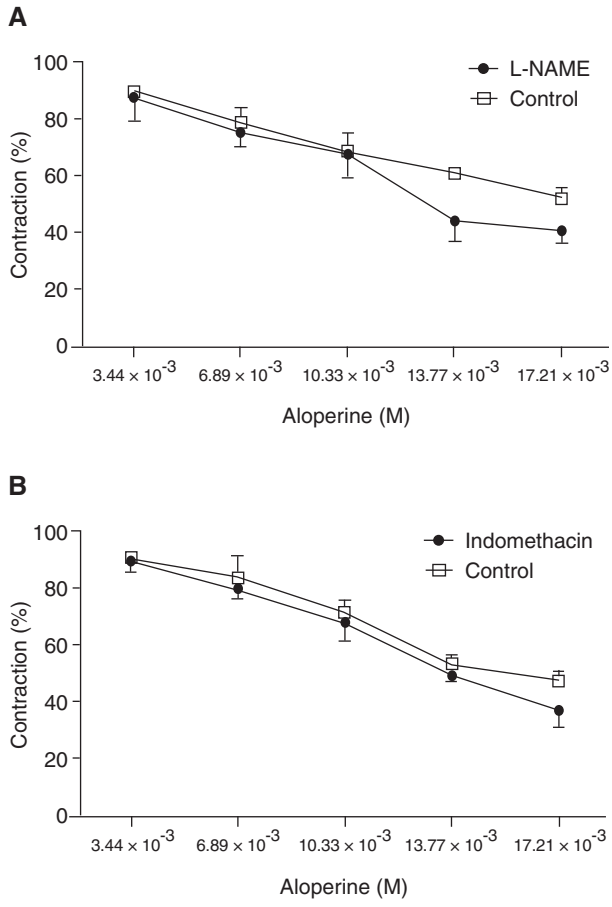

Fig. 5. Respective effects of (A) L-NAME and (B) indomethacin on vasorelaxation action of aloperine in rat thoracic aortic rings (n = 6 per group). Data are expressed as the mean ± SEM.

Discussion

To our knowledge, this is first study has to show that aloperine has vasodilatory effects on rat thoracic aortic rings. Recently, many studies have found that regulation of vasoreactivity plays a pivotal role in treatment in cardiovascular disease (10, 34, 41, 44). Therefore, the present study was designed to investigate vasorelaxant effects of aloperine by using aortic rings. Rat aorta ring assay were widely selected in the pharmacological study, becuase it provides an effective, adaptable, cheap and rapid method of cardiovascular research. More importantly, organic structures of rats and genotype are similar to human so that basic research is significant for clinical application (27, 32). Currently, the two compounds which commonly used to construct a pre-contracted model of thoracic aortic rings are KCl and PE (1, 18, 20, 29). However, the mechanisms are different. It is generally believed that the opening of the two calcium (Ca^{2+}) channels in artery involved in vasoconstriction: voltage-operated Ca^{2+} channels (VOCC) and receptor operated Ca^{2+} channel (ROCC). The ROCC is the main target of PE. PE-induced vasoconstriction is caused by the activation of the α1-adrenergic receptor, fol-

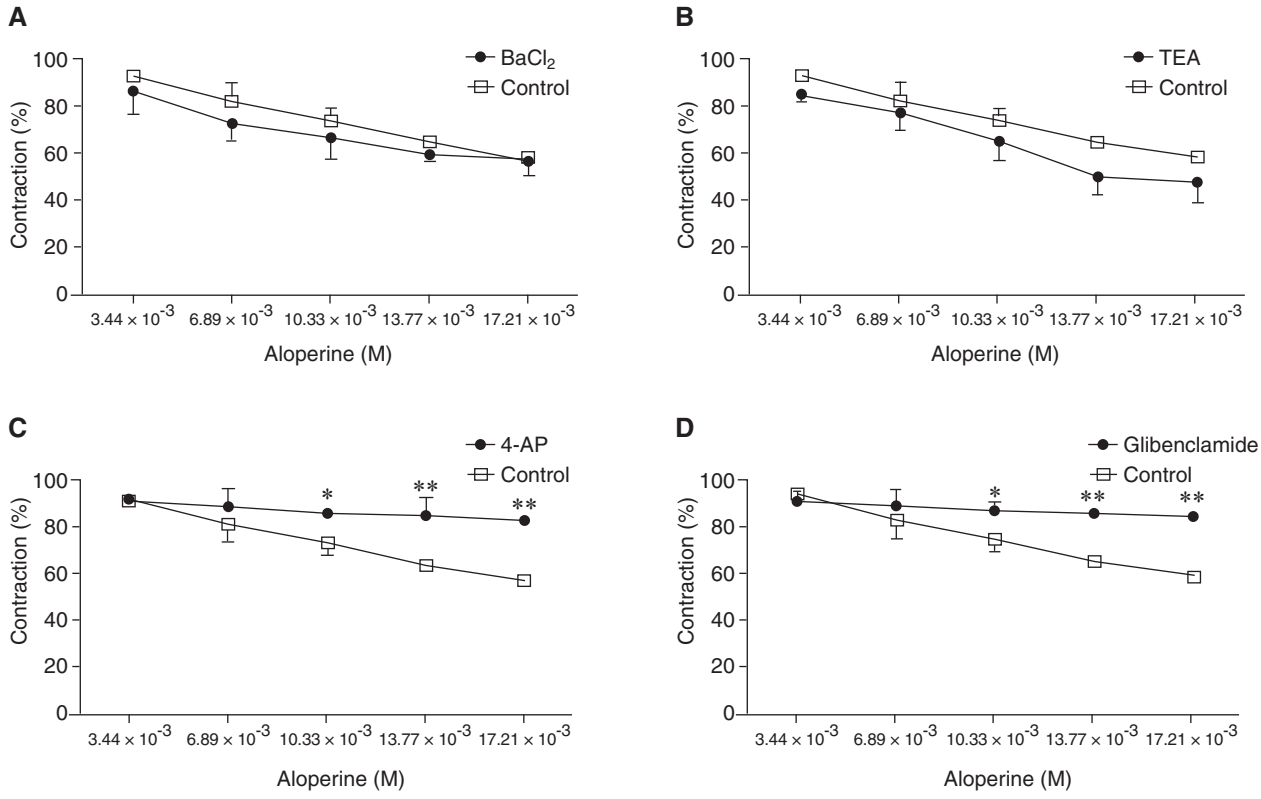

Fig. 6. Respective effects of (A) $BaCl_2$, (B) TEA, (C) 4-AP and (D) glibenclamide on vasorelaxation action of aloperine in rat thoracic aortic rings (n = 6 per group). Data are expressed as the mean ± SEM. $*P < 0.05$, $**P < 0.01$ *vs.* the control group.

lowed by activation of phospholipase C to produce diacylglycerol (DAG) and 1,4,5-triphophate inositol (IP3). DAG activates myosin light chain (MLC) through protein kinase C (PKC), and IPs also can directly induce the release of calcium from the sarcoplasmic reticulum, which such two ways leading to vasoconstriction (6, 17, 21). Whereas, contractile responses were mediated by KCl occurs by opening VOCC to make calcium influx (8, 11). The findings in this study are demonstrated that aloperine causes relaxation on PE-induced contractions in rat aorta rings. In contrast, this vasoactivity was not observed in KCl-pretreated rings. It suggesting that aloperine exerts its relaxation effect by selectively interfering with ROCC but not VOCC.

At present, it is reported that vascular endothelium mainly possess mechanical barrier and secretion of vasoactive substances that regulating vascular state and other physiological functions (3, 25, 31). Endothelial cells regulate vascular tone by secreting a series of contractile and diastolic factors including NO and prostaglandins (PGs) (9, 25). NO is a potent endothelium-dependent vasodilator, which is generated from L-arginine under the catalysis of NO synthase (NOS). It can activate soluble guanylyl cyclase (sGC) in vascular smooth muscle cells, thus increasing concentration of cyclic guanosine phosphate (cGMP), which in turn inhibits protein kinase G phosphorylation, reduce the Ca^{2+} influx, and then relaxes the blood vessel. NOS inhibitors, L-NAME, can block this relaxation action (32, 37). Meanwhile, it is well known that prostaglandins (PGs) have important roles in vasodilatation activities. Previous studies reported that activated phospholipase A2 releases arachidonic acid (AA) under the stimulation of various kinds of factors. AA is catalyzed by cyclooxygenase (COX) and prostacyclin synthase to transform into PGs. Then, the vasorelaxation occurred after the combination of PGs with its specific receptor. This vasorelaxation is inhibited by indomethacin, a COX inhibitor (32, 33, 35). The results presented in this study reflect that aloperine had no effect on the tension of isolated rat thoracic aorta rings without any pretreatment applied, indicating that normal systolic and diastolic activity of vascular rings was not affected by this alkaloid. In the endothelium-intact aortic rings pre-contracted with PE, aloperine showed a concentration-dependent relaxation. However, this vasorelaxation action of aloperine in rings was not attenuated by removal of endothelium. Afterwards, vasodilatory effects of aloperine

Table 1. Several types of K^+ channels and their modulator.

Item	Substance/ligand
Blocking Ca^{2+}-activated potassium channels	TEA
Blocking voltage dependent potassium channels	4-AP
Blocking inward rectifier potassium channels	$BaCl_2$
Blocking ATP-sensitive potassium channels	Glibenclamide

were detected after treated with two blockers of endothelium-dependent vasorelaxation, L-NAME and indomethacin. The data demonstrated that the two blockers did not influence effects of aloperine on the tension of the vascular rings. It is suggested that the vasodilative mechanism of this alkaloid may be non-endothelium dependent.

In this work, we have also investigated the role of K^+ channel on vasorelaxation induced by aloperine in rat thoracic aortic rings. Vasodilator drugs can be divided into two categories. endothelium-dependent agent and endothelium-independent agent (24). Wherein, endothelium-independent vasodilatation effects are related to K^+ channel (19). Excepting to maintaining resting membrane potential, K^+ channels also affect the calcium channel opening and closing (10, 23, 38, 39). Blockade of K^+ channels causes cell membrane depolarization, leading to increased Ca^{2+} influx through Ca^{2+} channels and subsequently increased vasoconstriction. In contrary, opening K^+ channels can hyperpolarize cell membrane, leading to closure of Ca^{2+} channels and decreased Ca^{2+} influx, then vasodilation is occurred. Currently, several distinct types of K^+ channels have become the focus of research: Ca^{2+}-activated potassium channels, voltage dependent potassium channels, inward rectifier potassium channels and ATP-sensitive potassium channels. These four K^+ channels are modulated respectively by TEA (2, 18), 4-AP (36), $BaCl_2$ (26, 28) and glibenclamide (5, 18) (Table 1).

This study showed that the effects of aloperine were attenuated by respective pretreatment with 4-AP and glibenclamide. Opposed to this phenomenon, TEA and $BaCl_2$ failed to interfere with such effect. Therefore, the results indicate that activation of K_v and K_{ATP} channels is likely involved in the vasorelaxant action of aloperine in isolated rat thoracic aorta rings. However, some studies suggested that $BaCl_2$ may nonspecific affect channels other than inward rectifier potassium channels (7), we would further study other possibilities in the next step.

In summary, the findings of present study provide evidence for the first time that aloperine has an endothelium-independent vasodilator effects in rat thoracic aortic rings. The possible mechanisms are inhibition of extracellular Ca^{2+} influx *via* the ROCC and opening K_v and K_{ATP} channels in aortic rings. Although the further investigations are needed, this study supports the opinion that this alkaloid is helpful for treatment of cardiovascular disease.

Acknowledgments

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Conflict of Interests

The authors declare that there are no conflicts of interests.

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