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Original Article

Biphasic effects of sodium danshensu on vessel function in isolated rat aorta

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Aim: To investigate the effects of sodium danshensu on vessel function in isolated rat aortic ring.
Methods: Thoracic aortae from normal rats were isolated and equilibrated in organ bath with Krebs-Henseleit buffer and ring tension was recorded. Effects of sodium danshensu on basal tonus of the vessel and its effects on vessel contraction and relaxation with or without endothelium were observed.
Results: In thoracic arteries under basal tonus, sodium danshensu (0.3–3 g/L) produced a dose-dependent transient contraction. In phenylephrine-precontracted thoracic arteries with or without endothelium, low concentration (0.1–0.3 g/L) of sodium danshensu produced a weak contraction, while high concentrations (1–3 g/L) produced a pronounced vasodilator after a transient vasocontraction. Pre-incubation with sodium danshensu could inhibit vessel contraction induced by phenylephrine and potassium chloride in a concentration-dependent way. Sodium danshensu inhibited phenylephrine- and $CaCl_2$-induced vasoconstriction in Ca^{2+}-free medium. Pre-incubation with tetraethylammonium, a non-selective K^+ channel blocker, and apamin, a small-conductance calcium-activated K^+ channel blocker partially antagonized the relaxation response induced by sodium danshensu. However, iberiotoxin (big-conductance calcium-sensitive K^+ channel blocker), barium chloride (inward rectifier K^+ channel blocker), and glibencalmide (ATP-sensitive K^+ channel blocker) had no influence on the vasodialtion effect of sodium danshensu.
Conclusion: Sodium danshensu showed a biphasic effects on vessel tension. While low dosage of sodium danshensu produced small contraction possibly through transient enhancement of Ca^{2+} influx, high dosage produced significant vasodilation mainly through promoting the opening of non-selective K^+ channels and small-conductance calcium-sensitive K^+ channels in the vascular smooth muscle cells.

Keywords: isolated aorta rings; sodium danshensu; vessel contraction and relaxation

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Introduction

Sodium danshensu [sodium *D*-(+)-β-(3,4-dihydroxyphenyl) lactate, $C_9H_9O_5Na$, molecular weight 220, Figure 1] is a sodium salt of danshensu from the widely used Chinese herb Danshen (*Salvia miltiorrhiza Bunge*). As a traditional Chinese medicine, Danshen is mainly used for the treatment of cardiovascular diseases such as angina pectoris, myocardial infarction and stroke[1]. Danshensu is a water-soluble phenolic acids component in Danshen. *In vitro* and *in vivo* studies revealed that danshensu possessed diverse pharmacological effects, including relaxation of coronary artery, anticoagulation, protection of myocardial ischemia-reperfusion injury, and antiarrhythmia activities[2–6]. However, the effects of sodium danshensu on vessel function in isolated rat aorta are not well demonstrated. The present study was aimed to elucidate the effects of sodium dansh-

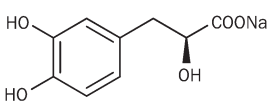

Figure 1. Chemical structure of sodium danshensu

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ensu on contraction and relaxation at various concentrations in rat thoracic aorta and the underlying mechanisms involved.

Materials and methods

Procedure for isolation of sodium danshensu

The root and rhizome of Salvia miltiorrhiza Bunge were collected in Hebei Province of China, and identified by Prof Hanchen ZHENG from the Department of Pharmacognosy, School of pharmacy, Second Military Medical University, Shanghai, China. A voucher specimen has been deposited in the herbarium of the School of pharmacy, Second Military Medical University, Shanghai, China. Danshensu was isolated from the root and rhizome of *Salvia miltiorrhiza Bunge*. Briefly, the dried and powdered roots and rhizomes (1.0 kg) were extracted under thermal reflux for 2 h with H_2O (12 L) accommodated to pH 9.0 with sodium hydroxide. The aqueous solution was adjusted to pH 2.0 with concentrated hydrochloric acid and subjected to macroporous adsorptive resin (HP20) and eluted with deionized water. The eluent was detected by thin layer chromatography (TLC) and the fraction contained danshensu was collected and evaporated under vacuum to a volume of 100 mL. After accommodated to pH 7.0 with sodium hydroxide, the concentrated solution was purified by recrystallization in 100 mL 50% acetone. Sodium danshensu was obtained as a white needle crystal, mp 255–258 °C. The purity of sodium danshensu was 99.7% by high performance liquid chromatography (HPLC) analysis.

Preparation of aortic rings

All experimental procedures were performed under protocols approved by the Animal Care Committee of the Animal Center at the Chinese Academy of Sciences in Shanghai, China. Male Sprague-Dawley rats (weighing 200–250 g) were anaesthetized by injecting with chloral hydrate (300 mg/kg) intraperitoneally. The thoracic aorta was removed carefully, cleaned of fat and adherent connective tissues in ice-cold Krebs-Henseleit (KH) buffer (pH 7.4) containing (in mmol/L): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.0, Na_2-EDTA 0.5. The aorta was cut into segments of 2–3 mm in length and then mounted onto two stainless-steel stirrups immersed in a 10-mL organ chamber, containing Krebs-Henseleit buffer that was gassed continuously with 95% O_2 and 5% CO_2, and maintained at 37 °C. One stirrup was connected to an isometric force transducer for tension measurement and recoding with a data-acquisition system (MPA 2000; Alcott Biotech, Shanghai, China).

The rings were stretched to a resting tension of 2.0 g and allowed to achieve equilibrium for 60 min with the bath fluid being changed every 15 min. In some rings, the endothelium was removed mechanically by inserting a polyethylene tube into the lumen of the ring and rolling the vessel with the tube gently onto moistened gauze. Endothelial integrity was assessed by degree of relaxation caused by acetylcholine (1 μmol /L) in the presence of contractile tone induced by phenylephrine (PE) (1 μmol /L). For the studies of endothelium-intact vessels, the ring was discarded if relaxation with acetylcholine was less than 80%. For studies of endothelium-denuded vessels, the rings were discarded if there was any degree of relaxation.

Effect of sodium danshensu on the basal tonus of aortic ring

After equilibration, endothelium-denuded rings were incubated with various concentrations of sodium danshensu (0.3, 1, or 3 g/L) respectively for 30 min, and the time-response curves for sodium danshensu was determined.

Effects of Ca^{2+}-free solution on the vasoconstriction of sodium danshensu

In order to investigate whether Ca^{2+} influx was related to the vasoconstriction of sodium danshensu, the normal Krebs-Henseleit solution was replaced for a Ca^{2+}-free solution containing EGTA (1 mmol/L), and endothelium-denuded rings were incubated with sodium danshensu (0.3, 1, or 3 g/L) for 30 min to obtain the time-response curves at various concentrations of sodium danshensu.

Effect of sodium danshensu on thoracic aorta rings pre-contracted with phenylephrine

After achieving equilibrium, phenylephrine (1 μmol/L) evoked a steady contraction in endothelium-intact and endothelium-denuded rings, and then, sodium danshensu (0.3, 1 or 3 g/L) was added respectively. The tension of each aorta ring was observed and recorded for 30 min after drug administration.

Effect of sodium danshensu on contractions induced by phenylephrine and KCl

After a 30-min incubation period with various concentrations of sodium danshensu (0.3, 1, or 3 g/L), cumulative concentration-response curves for phenylephrine (ranging from 1 nmol/ L to 100 μmol/ L) and KCl (ranging from 10 mmol/L to 90 mmol/L) were determined in endothelium-denuded rings.

Effect of sodium danshensu on contraction induced by $CaCl_2$

To assess the effects of sodium danshensu on $CaCl_2$-induced contractions, in this group of experiment, only one response is obtained with each vessel ring. Endothelium-denuded rings were first contracted with phenylephrine (1 μmol/L) to deplete the intracellular Ca^{2+} stores in Ca^{2+}-free solution containing 1 mmol/L ethyleneglycoltetraacetic acid (EGTA) for approximately 60 min. The rings were then rinsed in Ca^{2+}-free solution without EGTA containing 30 mmol/L KCl. At this time, if $CaCl_2$ was added, $CaCl_2$-induced vasoconstriction is through Ca^{2+} influx from the extracellular medium. The contractions induced by $CaCl_2$ (0–2.5 mmol/L) were obtained in the absence of sodium danshensu (control group) or after a 30-min period incubation with sodium danshensu (0.3, 1, or 3 g/L).

Effect of sodium danshensu on Ca^{2+} release from intracellular stores sensitive to phenylephrine

In order to investigate whether sodium danshensu could interfere with Ca^{2+} release from intracellular stores, the normal Krebs-Henseleit solution was replaced by a Ca^{2+}-free solution containing EGTA (1 mmol/L). The endothelium-denuded rings were exposed to Ca^{2+}-free solution for 15 min and then stimulated with phenylephrine 1 μmol/L. The contractions were obtained in the absence (control group) or after a 30-min incubation period with sodium danshensu (0.3, 1, or 3 g/L).

Influence of K^+ channel blockers on sodium danshensu-induced vasorelaxation

To further investigate the possible mechanisms responsible for sodium danshensu-induced relaxation, which was found to be non-endothelium dependent, endothellium-denuded rings were pre-incubated with one of the following K^+ channel blockers: tetraethylammonium (TEA, 10 mmol/L), iberiotoxin (0.1 μmol/L), glibenclamide (3 μmol/L), apamin (0.1 μmol/L), barium chloride ($BaCl_2$, 30 μmol/L), or a mixture of TEA (10 mmol/L) and apamin (0.1 μmol/L) for 10 min before phenylephrine was added, and when the contractile plateau was attained, sodium danshensu (3 g/L) was added. Since glibenclamide was prepared in dimethylsulfoxide (DMSO), a DMSO control group was used with the same volume in glibenclamide group.

Measurement of cGMP content in thoracic aorta

The vessel rings with endothelium were stretched to a resting tension of 2.0 g and allowed to be equilibrated for 30 min with the bath fluid being changed every 15 min. Sodium danshensu (0.3, 1, and 3 g/L) was added to the bath and incubated for 30 min. Then the tissues were weighed and homogenized in cold acetic acid (50 mmol/L, pH 4.75). The homogenates were centrifuged at 13000 *g* for 10 min and extracted twice with alcohol for 5 min. After centrifugation, the supernatant was collected in a glass bottle and dried for determination of cGMP using a radioimmunoassay kit (Shanghai Traditional Medicine University Medical Radiology Lab, Shanghai, China) according to the manufacturer's protocol.

Reagents

Phenylephrine hydrochloride, acetylcholine hydrochlorede, tetraethylammonium, iberiotoxin, apamin, glibenclamide, ruthenium red, ethyleneglycoltetraacetic acid (EGTA) and DMSO were all purchased from Sigma-Aldrich (St Louis, MO, USA). Verapamil hydrochloride injection was purchased from Shanghai Harvest pharmaceutical Co, Ltd. And phentolamine mesilate injection was purchased from Shanghai Sunrise Pharmaceutical Co, Ltd. Sodium danshensu was dissolved with Krebs-Henseleit solution. Glibenclamide was prepared as stock solution in DMSO. The other drugs were dissolved in distilled water. The bath concentration of distilled water or DMSO did not exceed 0.2%, which was shown to have no effect *per se* on the basal tonus of the preparations or on the agonist-mediated contraction or relaxation.

Statistical analysis

All results are expressed as mean±SEM. Statistical analysis was performed by unpaired Student's *t*-test for observations between two groups or by ANOVA for comparisons of multiple groups. A value of $P<0.05$ was considered statistically significant.

Results

Effect of sodium danshensu on the basal tonus

In normal Krebs-Henseleit solution, for aorta rings in basal tonus states, sodium danshensu first caused a contraction which achieved the maximum at about the eighth minute, and then followed by a spontaneous relaxation. The maximal contraction is 0.86±0.04 g, 1.46±0.03 g and 1.49±0.05 g for sodium danshensu 0.3 g/L, 1 g/L, and 3 g/L respectively (data are mean±SD, n=12–15 segments for each group, Figure 2A).

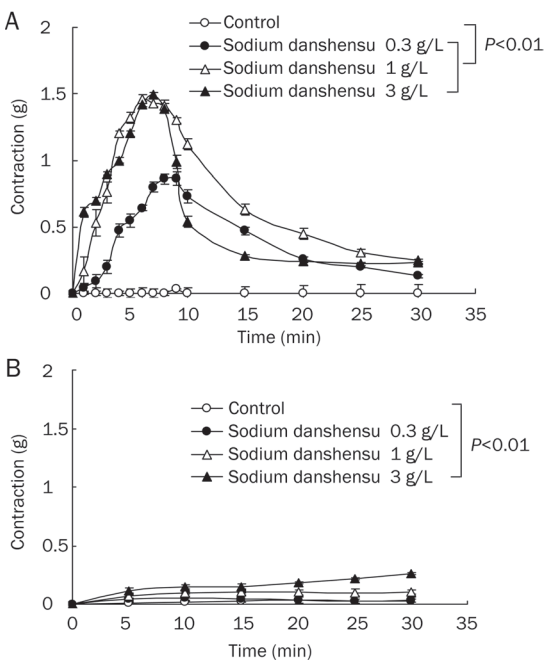

Figure 2. Effect of sodium danshensu on basal tonus in normal Krebs-Henseleit buffer (A) and in Ca^{2+}-free Krebs-Henseleit buffer containing 1 mmol/L ethyleneglycoltetraacetic acid (EGTA) (B).

In Ca^{2+}-free solution, the transient contraction induced by sodium danshensu was markedly reduced compared with that in normal Krebs-Henseleit solution, although not being deleted completely (Figure 2B).

Biphasic effect of sodium danshensu on PE-precontracted aorta

It can be seen in Figure 3A, in phenylephrine pre-contracted rat thoracic aorta, as compared with control (Figure 3Aa),

sodium danshensu caused small contraction at the concentrations ranging from 0.1 g/L to 0.3 g/L (Figure 3Ab and Figure 3Ac), but at the concentrations ranging from 1 g/L to 3 g/L (Figure 3Ad, Figure 3Ae, and Figure 3Af), sodium danshensu produced a biphasic response: a transient contraction followed by a pronounced relaxation. There was no difference in the contraction or vasodilation responses to sodium danshensu between vessel with and without endothelium (Figure 3B).

Effects of sodium danshensu incubation on PE- and KCl-induced contraction

The effects of sodium danshensu on the cumulative concentration-response curves for phenylephrine and KCl on isolated rat thoracic aorta are shown in Figure 4 and Figure 5. The tension for phenylephrine or KCl in endothelium-denuded rings were depressed in the presence of sodium danshensu.

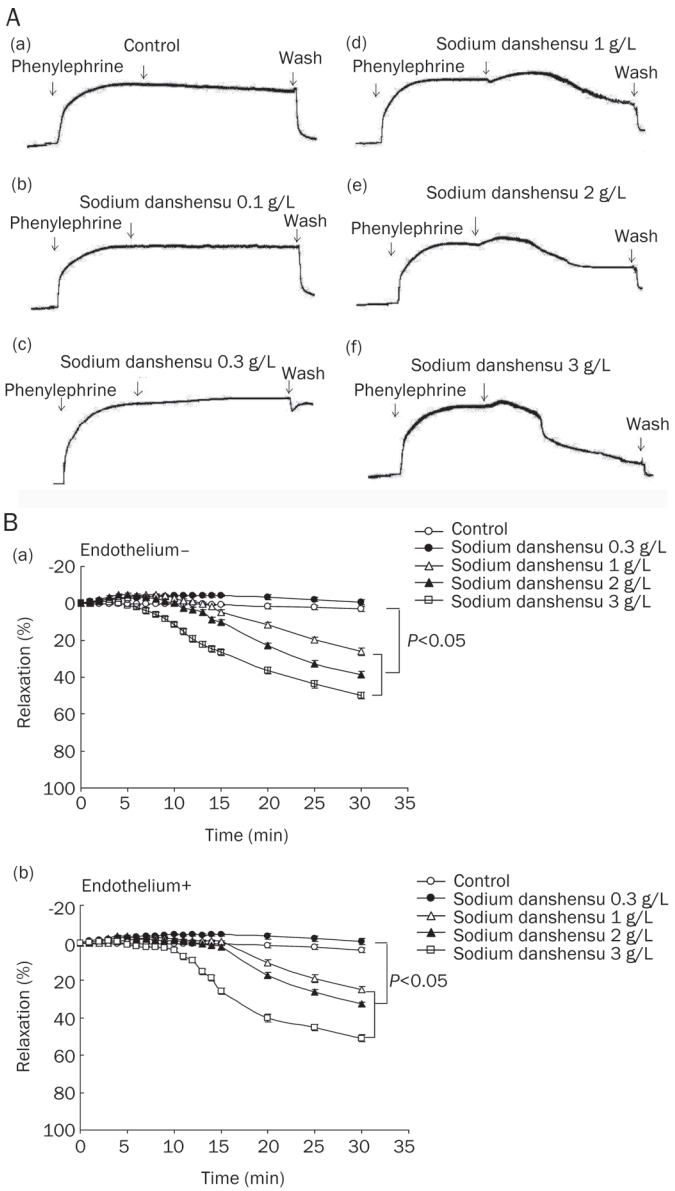

Figure 3. Representative tracing of vessel contraction and relaxation at different concentrations (A) and dose- and time-dependent vasoactive effects of sodium danshensu in aortic rings with and without endothelium (B).

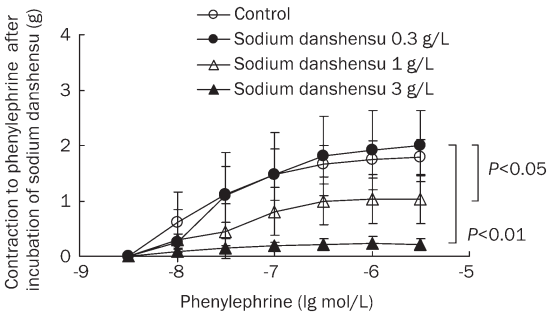

Figure 4. Effects of sodium danshensu on phenylephrine-induced contractile response in endothelium-denuded rat aortic rings. The responses were determined after 30-min pre-incubation with sodium danshensu. Data are mean±SD. *n*=6–7 segments.

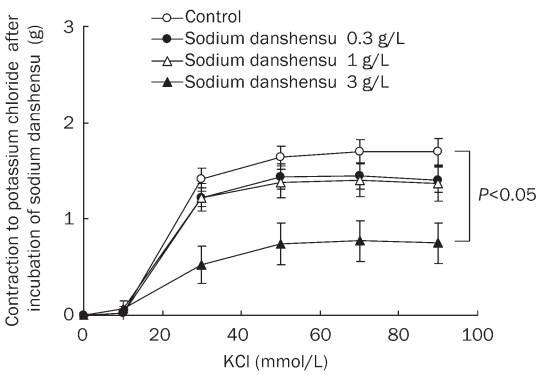

Figure 5. Effects of sodium danshensu on KCl-induced contractile response in endothelium-denuded rat aortic rings. The responses were determined after 30-min pre-incubation with sodium danshensu. Data are mean±SD. *n*=6–7 segments.

Effect of sodium danshensu on $CaCl_2$-induced contraction

Pretreatment with sodium danshensu attenuated $CaCl_2$-induced contraction of denuded rat aorta exposed to Ca^{2+}-free solution containing KCl. Preincubation of the rings with sodium danshensu at the contraction of 3 g/L significantly reduced the contraction induced by $CaCl_2$ (Figure 6).

Effect of sodium danshensu on intracellular Ca^{2+} release

The results presented in Figure 7 show that sodium danshensu significantly inhibited the contractions induced by phenylephrine in Ca^{2+}-free solution containing EGTA. In Ca^{2+}-free solution, the maximum contraction elicited by phenylephrine was 0.18±0.03 g in control group, and this was attenuated to 0.16±0.02 g, 0.12±0.03 g, and 0.09±0.01 g (50% reduction; *n*=5

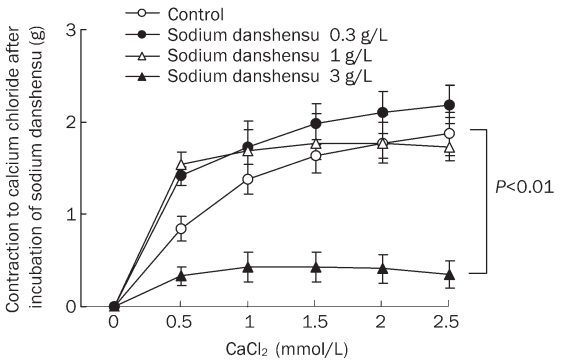

Figure 6. Effects of sodium danshensu on $CaCl_2$-induced contractile response in endothelium-denuded rat aortic rings. Contraction induced by $CaCl_2$ was determined in Ca^{2+}-free solution containing 30 mmol/L KCl after 30-min incubation with sodium danshensu (0.3, 1, and 3 g/L).

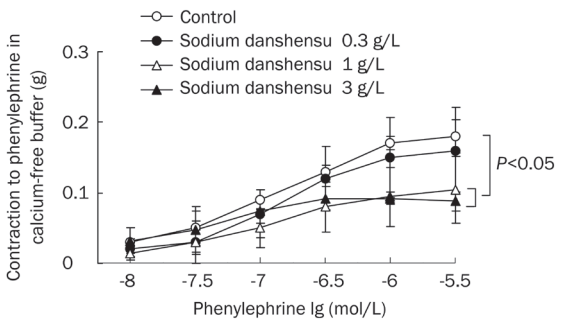

Figure 7. Effects of sodium danshensu on intracellular Ca^{2+} release sensitive to phenylephrine in aortic rings. Intracellular Ca^{2+} release sensitive to phenylephrine was examined by contraction the rings with phenylephrine after exposure for 1 min to calcium-free medium after 30-min incubation with sodium danshensu (0.3, 1, and 3 g/L).

for each group; $P<0.05$) in the presence of sodium danshensu 0.3 g/L, 1 g/L, and 3 g/L, respectively.

Influence of potassium channel blockers on sodium danshensu-induced vasorelaxation

In order to examine the role of specific types of K^+ channels in the sodium danshensu-induced response, aortic rings were incubated with either TEA (10 mmol/L), iberiotoxin (0.1 μmol/L), glibenclamide (3 μmol/L), apamin (0.1 μmol/L), barium chloride (30 μmol/L), or a mixture of TEA (10 mmol/L) and apamin (0.1 μmol/L) for 10 min prior to the application of phenylephrine. Sodium danshensu (3 g/L) was added after the contraction to phenylephrine reached a plateau and vessel tension was observed for 30 min. In the presence of TEA (a non-selective K^+ channel blocker), vasorelaxation effect of sodium danshensu was partially inhibited. Similarly, apamin (a small-conductance K_{Ca} channel blocker) also caused a significant inhibition of relaxation partially. The combination of the two kinds of K^+ channel inhibitors consisted of TEA (10 mmol/L) and apamin (0.1 μmol/L) also produced partial inhibition of relaxation significantly. However, iberiotoxin (a big-conductance calcium-sensitive K^+ channel blocker), barium chloride (a inward rectifier K^+ channel blocker) and glibencalmide (a ATP-sensitive K^+ channel blocker) did not affect the relaxation effect of sodium danshensu (Figure 8).

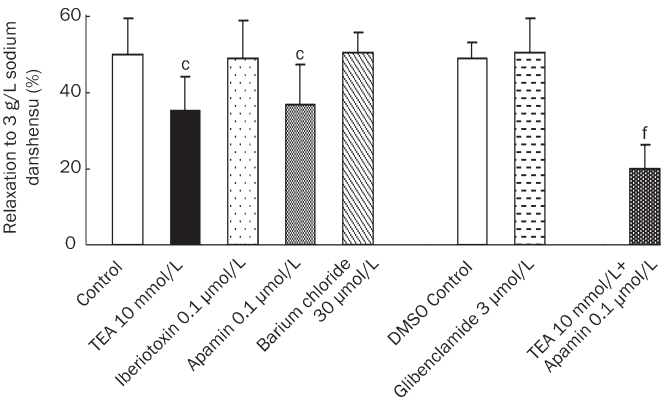

Figure 8. Effects of different K^+ channel blockers on sodium danshensu (3 g/L)-induced relaxation in 1 μmol/L phenylephrine-precontracted endothelium-denuded rat thoracic aortic rings. Each individual blocker was pre-incubated with the rat aorta for 10 min prior to stimulation with 1 μmol/L phenylephrine. Sodium danshensu (3 g/L) was added after the contraction to phenylephrine reached plateau. Non-selective K^+ channel blocker tetraethylammonium (TEA, 10 mmol/L) and small-conductance Ca^{2+}-activated K^+ channel blocker apamin (0.1 μmol/L) significantly partially reduced sodium danshensu-induced relaxation, while big-conductance Ca^{2+}-activated K^+ channel blocker iberiotoxin (0.1 μmol/L), inward rectifier K^+ channel blocker barium chloride ($BaCl_2$, 30 μmol/L) and ATP-sensitive K^+ channel blocker glibencalmide (3 μmol/L) did not alter the relaxant effect of sodium danshensu. The combination of non-selective K^+ channel blocker TEA (10 mmol/L) and small-conductance Ca^{2+}-activated K^+ channel blocker apamin (0.1 μmol/L) also produced significant inhibition partially, but not entirely. $^cP<0.01$ *vs* control. $^fP<0.01$ *vs* TEA 10 mmol/L.

Effect of sodium danshensu on the concentration of cGMP in rat thoracic aorta

The content of cGMP in rat thoracic aorta with endothelium was significantly depressed after being incubated with sodium danshensu (Figure 9). This result indicated the role of the cGMP pathway in the vasoconstriction effect of sodium danshensu.

Discussion

The present study revealed a biphasic response to sodium danshensu in both basal-equilibrated and contracted thoracic arteries. In basal tonus aortic rings, sodium danshensu produced a transient contraction followed by a spontaneous vasodilator response in endothelium-denuded aortic rings. In phenylephrine pre-contracted thoracic arteries, low concentration of sodium danshensu produced a small contraction, and sodium danshensu in high concentrations produced transient small contractions followed by significant vasodila-

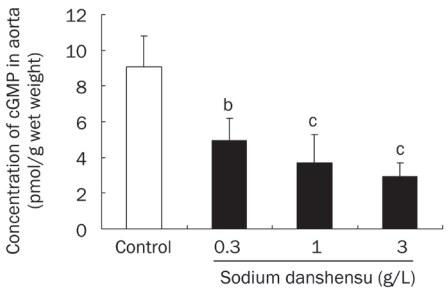

Figure 9. Effect of sodium danshensu on the content of cGMP in rat thoracic aorta with endothelium. The content of cGMP in rat thoracic aorta with endothelium was significantly depressed after being incubated with sodium danshensu, in a dose-dependent manner. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control.

tor responses in both endothelium-intact and endothelium-denuded vessels.

It has been reported that danshensu at the concentration of 0.001 mg/mL could relax isolated swine coronary artery and inhibit the vasoconstriction induced by morphine and propranolol[2]. Their result of relaxation effect is similar to ours but they did not report contraction response. In a previously report, danshen capsule had been demonstrate a biphasic response in rat femoral arteries: at low concentrations it caused small constrictions, but at high concentrations it produced marked vasodilatation. Our results revealed that sodium danshensu had a similar effect to danshen capsule reported[7]. However, the chemical components of danshen capsule are multiple and it is not clear which component is responsible for the phenomenon. In the present study we used pure sodium danshensu and the findings indicate that sodium danshensu may be the main component that induced the biphasic response among the component from danshen.

Mechanisms in sodium danshensu-induced vessel contraction

There are several possible mechanisms involved in the sodium danshensu-induced contraction of vascular smooth muscle. In the present study, the transient extracellular Ca^{2+} influx may play an important role in sodium danshensu-mediated vasoconstriction responses. In Ca^{2+}-free solution, the contraction induced by sodium danshensu was markedly reduced compared with that in normal Krebs-Henseleit solution (Figure 2B). It indicated that extracellular Ca^{2+} influx induced by sodium danshensu may be important in eliciting the transient vasoconstriction.

In the present study, sodium danshensu incubation also reduced the content of cGMP level in rat thoracic aorta with endothelium (Figure 9), which may add to the contraction effect in the basal condition. It is well known that cGMP can active intracellular protein kinases G and then lead to vasodilation. cGMP synthesis is catalyzed by soluble guanylate cyclase (sGC), which converts Gtp to cGMP, and numerous cyclic nucleotide phosphodiesterases (PDE) can degrade cGMP by hydrolyzing cGMP into 5′-GMP. However, whether sodium danshensu reduced the content of cGMP in aorta through inhibiting soluble guanylate cyclase (sGC) to suppress the cGMP synthesis or activing phosphodiesterases needs further investigation. In any case, generally speaking, the inhibition of cGMP may produce a stable contraction, but it showed an unstable contraction in the present study (Figure 2A). This could be explained as that, since the effect of sodium danshensu is biphasic, except the factors of promoting vessel contraction, the factors of inducing vessel dilation may exist at the same time, and one of the two kinds of factors may play a leading role in different period. The unstable contraction may further consolidate the view that the effect of sodium danshensu is biphasic in another aspect.

Endogenous release of calcium may not be involved in the contraction response since large dose of sodium danshensu actually inhibited phenlylephrine-induced contaction, indicating inhibition of endogenous release of calcium. Neither ruthenium red (an inhibitor of Ca^{2+} release from sarcoplasmic reticulum) nor phentolamine (a nonselective alpha-adrenergic antagonist) showed any reduction for sodium danshensu induced contraction (data not shown). Therefore, it appears that the transient contraction is not mediated by Ca^{2+} release from intracellular stores or activation of alpha-adrenergic receptor.

Mechanisms in sodium danshensu-induced vessel dilation

Sodium danshensu in high concentrations constantly produced a significant vasodilation response after transient contraction, whether the endothelium is removed or not, indicating smooth muscle dependent vasodilation.

Different mechanisms may be involved in sodium danshensu-induced vessel relaxation. Sodium danshensu may exert vasodilation through inhibiting extracellular calcium influx and phenylephrine-sensitive endogenous calcium release, as we found that preincubation of sodium danshensu could inhibit calcium-induced vasoconstriction in endogenous calcium-depleted state as well as phenylephrine-induced contraction in calcium-free buffer. This is in consistent with the report that danshensu produced vasodilator action in isolated rat coronary arteries at a concentration range of 0.02 to 0.035 mg/mL, mainly by inhibition of Ca^{2+} influx[3]. Phenylephrine and potassium chloride induced contraction are both through increasing intracellular Ca^{2+} concentration but with different cellular mechanisms. Phenylephrine activates the receptor-operative Ca^{2+} channel and induces an influx of extracellular Ca^{2+}. Simultaneously, phenylephrine mobilizes Ca^{2+} release from intracellular stores through binding toα_1-adrenoceptor and stimulating phospholipase C or the specific IP_3 receptor (IP_3R) channel in sarcoplasmic reticulum (SR) membrane[8]. Potassium chloride induces Ca^{2+} influx through voltage-dependent Ca^{2+} channel, which further activate Ca^{2+}-induced Ca^{2+} release through a ryanodine receptor[9].

In Ca^{2+}-free medium containing EGTA, phenylephrine-

induced vasoconstriction is through activing the IP_3R channel in sarcoplasmic reticulum membrane and inducing the release of intracellular Ca^{2+} release from sarcoplasmic reticulum[10, 11]. In our study, sodium danshensu dose-dependently reduced phenylephrine-induced contraction in Ca^{2+}-free medium, demonstrating that sodium danshensu can reduce Ca^{2+} release from intracellular stores sensitive to phenylephrine[12]. After aortic rings first were contracted with phenylephrine to deplete the intracellular Ca^{2+} stores in Ca^{2+}-free medium containing EGTA and then rinsed in Ca^{2+}-free medium (without EGTA) containing KCl, $CaCl_2$-induced vasoconstriction is through Ca^{2+} influx from the extracellular medium[12]. In the present study, $CaCl_2$-induced vasoconstriction in Ca^{2+}-free medium containing KCl was inhibited significantly by sodium danshensu, indicating that sodium danshensu behaved as a non-selective calcium antagonist. This is in agreement with a recent study with rat coronary artery[3]. Taken together, these results demonstrated that sodium danshensu could block Ca^{2+} influx and reduce Ca^{2+} release from intracellular stores sensitive to phenylephrine.

Direct activation of K^+ channels on arterial smooth muscle cells normally hyperpolarizes the cell membrane, inhibits Ca^{2+} influx through voltage-operative Ca^{2+} channel, and suppresses smooth muscle contraction[13–15]. To further explore whether a particular K^+ channels is involved in the sodium danshensu-induced vasorelaxation in high concentrations, various of potassium channel blockers was used as tools in the present study. TEA, a non-selective K^+ channel blocker, significantly inhibited the vasorelaxation partially. Apamin, a small-conductance K_{Ca} channel blocker, also caused partial inhibition of the sodium danshensu-induced relaxation. However, big-conductance calcium-sensitive K^+ channel inhibitor iberiotoxin did not alter the relaxation effect of sodium danshensu, which is in accordance with a previous finding on porcine coronary artery smooth muscle cells[16]. Similarly, the blockade of inward rectifier K^+ channel and ATP-sensitive K^+ channel did not affect the relaxation response of sodium danshensu. These findings indicated that activation of non-selective K^+ channel and small-conductance calcium-sensitive K^+ channel in the vascular smooth muscle cells may also contribute to vasorelaxation to some extent. Because either non-selective K^+ channel blocker TEA or small-conductance K_{Ca} channel blocker apamin produced only partial inhibition, did not abolish the vasodilator of sodium danshensu, the mixture of the two kinds of K^+ channel inhibitors were tested. The mixture of the two kinds of K^+ channel inhibitors also could not abolish the vasodilator of sodium danshensu entirely though could produce significant inhibition partially. This further consolidates the view that except direct opening of K^+ channels, there are other pathways for sodium danshensu producing the vessel dilation.

As the concentration of sodium danshensu induced vasodilation is very high, nonspecific action could not excepted.

In conclusion, the present study has indicated that sodium danshensu showed a biphasic effect in isolated rat thoracic aorta rings. While low contractions of sodium danshensu produced small vasoconstrictor responses by enhancing transient extracellular Ca^{2+} influx and reducing the vessel content of cGMP, high concentrations of sodium danshensu produced pronounced vasodilator responses through blocking calcium influx and inhibiting intracelluar calcium release, as well as opening non-selective K^+ channel and small conductance calcium-sensitive K^+ channels in the vascular smooth muscle cells. The calcium influx promotion and inhibition induced by sodium danshensu during contraction and relaxation may involve different channels and needs further exploration, and using an electrophysiological method to record the Ca^{2+} current may assist to interpret this phenomenon.

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Author contribution

Ning ZHANG, Chuan ZHANG and Hong CHEN designed the research; Ning ZHANG, Mei-fang ZHONG, Hao ZOU, Jian WANG and Lei JIN performed the research; Chuan ZHANG and Hong CHEN contributed new reagents and analytical tools; Peng HUANG and Bing-qing GU carried out the preliminary experiment; Ning ZHANG analyzed data; Ning ZHANG wrote the paper; Chuan ZHANG, Hong CHEN, and Shi-long MAO revised the paper.

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