Relaxant Effects of Matrine on Aortic Smooth Muscles of Guinea Pigs[1]

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Objective To determine whether matrine, a kind of traditional Chinese medicinal alkaloid, can relax the aortic smooth muscles isolated from guinea pigs and to investigate the mechanism of its relaxant effects. **Methods** Phenylephrine or potassium chloride concentration-dependent relaxation response of aortic smooth muscles to matrine was studied in the precontracted guinea pigs. **Results** Matrine (1×10^{-4} mol/L -3.3×10^{-3} mol/L) relaxed the endothelium-denuded aortic rings pre-contracted sub-maximally with phenylephrine, in a concentration-dependent manner, and its pre-incubation (3.3×10^{-3} mol/L) produced a significant rightward shift in the phenylephrine dose-response curve, but had no effects on the potassium chloride-induced contraction. The anti-contractile effect of matrine was not reduced by the highly selective ATP-dependent K^+ channel blocker glibenclamide (10^{-5} mol/L), either by the non-selective K^+ channel blocker tetraethylammonium (10^{-3} mol/L), or by the β-antagonist propranolol (10^{-5} mol/L). In either "normal" or "Ca^{2+}-free" bathing medium, the phenylephrine-induced contraction was attenuated by matrine (3.3×10^{-3} mol/L), indicating that the vasorelaxation was due to inhibition of intracellular and extracellular Ca^{2+} mobilization. **Conclusion** Matrine inhibits phenylephrine-induced contractions by inhibiting activation of α-adrenoceptor and interfering with the release of intracellular Ca^{2+} and the influx of extracellular Ca^{2+}.

Key words: Matrine; Aorta; Vascular smooth muscle; Relaxation; Guinea-pigs; Calcium

INTRODUCTION

Matrine, a major quinoilizidine alkaloid with four-loop and molecular formula of $C_{15}H_{24}N_2O$ (Fig. 1), is extracted from *Sophora alopecuroides L*, a Chinese medicinal plant, and has been intensively studied for its pleiotropic effects on cardiovascular diseases[1]. It has been shown that matrine inhibits ventricular arrhythmia induced by aconitine, barium chloride or coronary ligation in rats, and reduces the incidence of atrium fibrillation produced by calcium chloride-acetylcholine mixture in mice[2-4]. The mechanism of matrine's anti-arrhythmic action might be associated with the prolonged action potential by blocking multiple potassium currents and by increasing intracellular calcium through promoting extracellular calcium flow into intracellular calcium, and has nothing to do with the release of intracellular calcium from ventricular myocytes[5-9]. Matrine can prevent myocardial hypertrophy and pathologic switching of myosin heavy chain isoform mediated by norepinephrine, thus reversing cardiac remodeling induced by norepinephrine, and so inhibits cardiac fibrosis stimulated by angiotensin II by suppressing cultured neonatal cardiac fibroblast proliferation and collagen synthesis[10-11]. It was reported that matrine inhibits angiotensin II-induced vascular smooth muscle cell proliferation and calcium overload[12-13]. We recently reported that the protective effect of matrine on myocardial infarction in hypercholesterolemia rats was due to its ability to decrease the total serum cholesterol and triglycerin level, to enhance the activities of anti-oxidative enzymes, and to maintain the stability of myocardial cellular membranes[14] (Fig. 1).

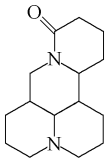

FIG. 1. Chemical structure of matrine.

Since no report is available about the effect of

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matrine on vasomotion, the present study aims to investigate the relaxant effects of matrine on vascular smooth muscle in order to gain insights into its mode of action.

MATERIAL AND METHODS

Animals

Guinea pigs, weighing 250±30 g, were supplied by the Animal Center of Ningxia Medical University (Ningxia, China). The animals were housed individually in cages under hygienic conditions in a 12 h light and dark cycle at 22±3 ℃ and 45%±10% humidity, with free access to standard rat diet and water. The study was approved by the Animal Center of Ningxia Medical University.

Reagents

Matrine with a purity>99% was purchased from Ningxia Bauhinia Pharmaceutical Co., Ltd. Glibenclamide, tetraethylammonium, and acetylcholine were purchased from Sigma Chemical Co. Phenylephrine was purchased from Shanghai Harvest Pharmaceutical Co., Ltd., and propranolol from Changzhou Siyao Pharmaceutical Co., Ltd. Glibenclamide was initially dissolved in dimethyl sulphoxide and further diluted in Krebs' solution to the proper final concentration. The final concentration of dimethyl sulphoxide in the solution did not exceed 0.1%. Matrine, tetraethylammonium, acetylcholine, phenylephrine, and propranolol were dissolved in Krebs' solution.

Experimental Protocols

Guinea pigs were sacrificed by a blow on the back of head followed by exsanguination under running water. The thoracic aorta was rapidly isolated and cleaned from adherent connective tissues. When endothelium-denuded tissue was used, the endothelial lining was mechanically removed by gently rubbing against a syringe needle. Then the thoracic aorta was cut into 3-4 mm long rings. Several rings cut from the same artery were studied in parallel. The rings were suspended between two stainless steel L-shaped hooks in a 10 mL organ chamber containing warmed (37 ℃) and oxygenated (95% O_2 and 5% CO_2) Krebs' solution. The Krebs' solution contained the following compositions (in mmol/L): 119.0 NaCl, 25.5 $NaHCO_3$, 4.3 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, and 11.0 glucose. One hook was fixed at the organ bath while the other was connected to a force transducer. Rings were equilibrated for 60 min under 2 g of resting tension. During this time, the rings were washed with Krebs' solution every 15 min. The tension of rings was continuously monitored by Chengdu Taimen BL-420E+Data Acquisition & Analysis System (Chengdu, China) and recorded on computer. At the end of a 60-min equilibration period, the viabilities of vessel specimens were checked with phenylephrine (10^{-5} mol/L), and preparations developing a tension less than 1 g were discarded[15]. The absence of endothelium was assessed prior to experiment by treating a phenylephrine-precontracted aortic ring with acetylcholine (10^{-5} mol/L). Rings giving a 10% reduction in phenylephrine-induced tone when treated with acetylcholine were regarded as being endothelium-denuded[16].

Examination of Effect of Matrine on Vasoconstrictions with Phenylephrine or Potassium Chloride

The isolated aortic rings were exposed to matrine (3.3×10^{-3} mol/L) or the Krebs' solution for 15 min. Phenylephrine (1×10^{-7}-1×10^{-4} mol/L) or potassium chloride (1×10^{-2}-8×10^{-2} mol/L) was cumulatively added into the organ bath at a 10-12 min interval. The effect of matrine on isolated aortic rings was always detected in parallel.

Relaxant Effect of Matrine on Vascular Smooth Muscle of Aortic Rings

The experiment was performed to evaluate the effect of matrine on guinea pig aortic rings precontracted submaximally (70%-80%) with contractile agents, phenylephrine (10^{-5} mol/L) and potassium chloride (4×10^{-2} mol/L) acting by different mechanisms. Increasing concentrations of matrine (1×10^{-4}-3.3×10^{-3} mol/L) were administered cumulatively at 4-7 min interval when the contractions to phenylephrine or potassium chloride reached a plateau.

Examination of Effect of Gilbenclamide, Tetraethylammonium and Propranolol Pretreatments on Matrine-induced Vasorelaxation

The aortic rings were incubated with gilbenclamide (10^{-5} mol/L), tetraethylammonium chloride (10^{-3} mol/L) and propranolol (10^{-5} mol/L) for 30 min or 15 min before phenylephrine (10^{-5} mol/L) was administered. The role of K^+ channel and β-antagonist activation in matrine-induced vasorelaxation was evaluated.

Examination of Effect of Matrine on Calcium Flux

Four aortic rings, taken from the same animal, were separately put into a 10 mL tissue bath as previously described[16-17]. Two preparations were washed with modified Krebs' solution, into which calcium was omitted and 4×10^{-4} mol/L EDTA was

added (calcium-free). The other two preparations were washed with normal Krebs' solution. After 10-min incubation, submaximal contractions to phenylephrine (10^{-5} mol/L) (C1) occurred in the four preparations. When the contractions reached their peak, the preparations were washed three times with normal Krebs' solution and incubated for 10 min. Matrine (3.3×10^{-3} mol/L) was then added into one of the calcium-free and normal preparations for 10 min and vehicle was added into the control before the second transient contraction was evoked by phenylephrine (C2) (Fig. 4A). A ratio of the responses to phenylephrine was obtained ((C2/C1)×100%) (Fig. 4B).

Statistical Analysis

Data were expressed as $\bar{x} \pm s$. Statistical analysis was performed with ANOVA. $P<0.05$ was considered statistically significant.

RESULTS

Effect of Matrine on Cumulative Concentration-response Curve for Phenylephrine or Potassium Chloride

After pretreatment with matrine (3.3×10^{-3} mol/L), the concentration-response curve for phenylephrine was shifted rightward and the maximal rate of inhibition was reduced to 77.45%, whereas the concentration-response curve for potassium chloride was shifted otherwise and the maximal rate of inhibition remained unreduced (Fig. 2).

Effect of Matrine on Vascular Smooth Muscle of Aortic Rings

Matrine (1×10^{-4}-3.3×10^{-3} mol/L) relaxed the vascular smooth muscle of aortic rings in a concentration-dependent manner. The maximal relaxation induced by matrine was 58.3%. However, matrine (1×10^{-4}-3.3×10^{-3} mol/L) had no dilatation-effect on aortic rings (Fig. 3A).

Effect of Cumulative Concentration-response Curves for Gilbenclamide, Tetraethylammonium, and Propranolol on Matrine-induced Relaxation

The incubation of aortic rings with highly selective ATP-dependent K^+ channel blocker glibenclamide, non-selective K^+ channel blocker tetraethylammonium and β-antagonist propranolol failed to modify the relaxant effect of matrine (1×10^{-4}-3.3×10^{-3} mol/L). The maximal relaxation values for glibenclamide, tetraethylammonium, and propranolol was 56.3%±7.8%, 54.6%±6.3%, and 59.1%±11.8%, respectively (Fig. 3B-D).

Effect of Matrine on Phenylephrine-induced Contractions in "Ca^{2+}-free" and "Normal" Ca^{2+} Medium

Matrine (3.3×10^{-3} mol/L) significantly decreased the phenylephrine-induced contractions in both "normal" and "Ca^{2+}-free" Krebs' solutions. The ratios were 57.9%±30% (n=8) and 36.7%±17% (n=8), as compared with their controls (110.1%±24.6% *vs* 83.2%±27.1%, Fig. 4B).

DISCUSSION

In the present study, matrine pretreatment shifted the concentration-response curve for phenylephrine rightward, but exerted no effect on the concentration-response curve for potassium chloride. Additionally, matrine relaxed the vascular smooth muscle of aortic rings in a concentration-dependent manner. It is well known that the cellular mechanism of contraction involved in response to potassium

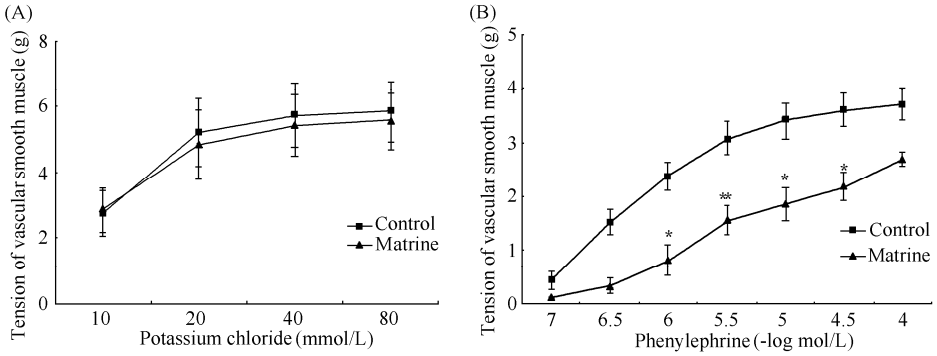

FIG. 2. Effect of matrine on aortic smooth muscle contraction curves for potassium chloride (A) and phenylephrine (B). The data are represented as $\bar{x} \pm s$ (n=8). $^{*}P<0.05$, $^{**}P<0.01$ *vs* control.

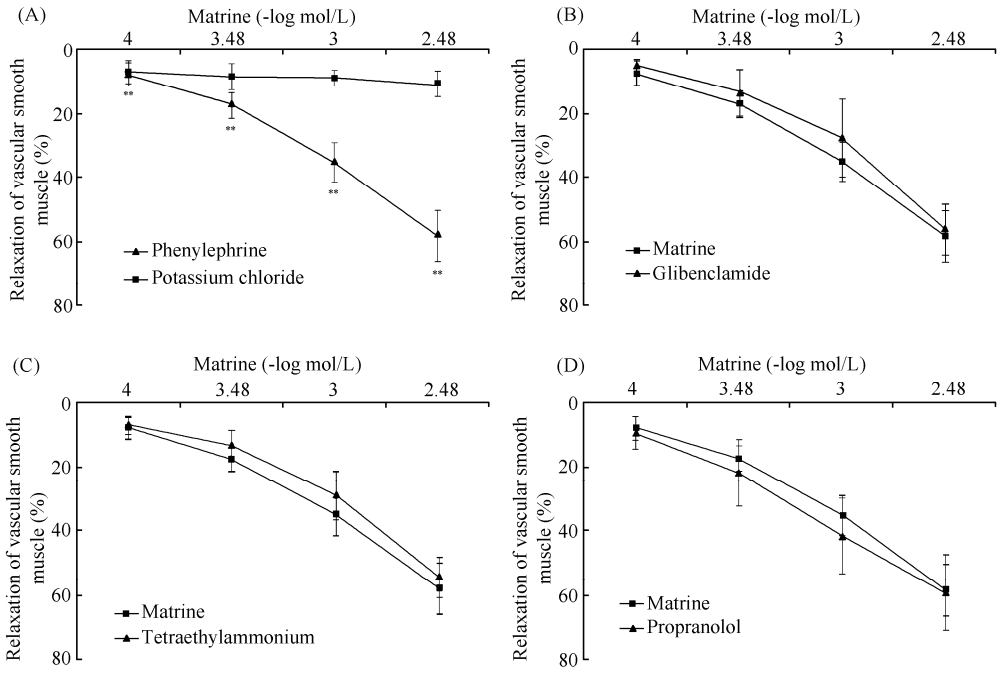

FIG. 3. Effects of matrine on aortic smooth muscle contractions induced phenylephrine and potassium chloride (A), glibenclamide (B), tetraethylammonium (C), and propranolol (D). The data are shown as $\bar{x} \pm s$, $^{**}P<0.01$ *vs* control.

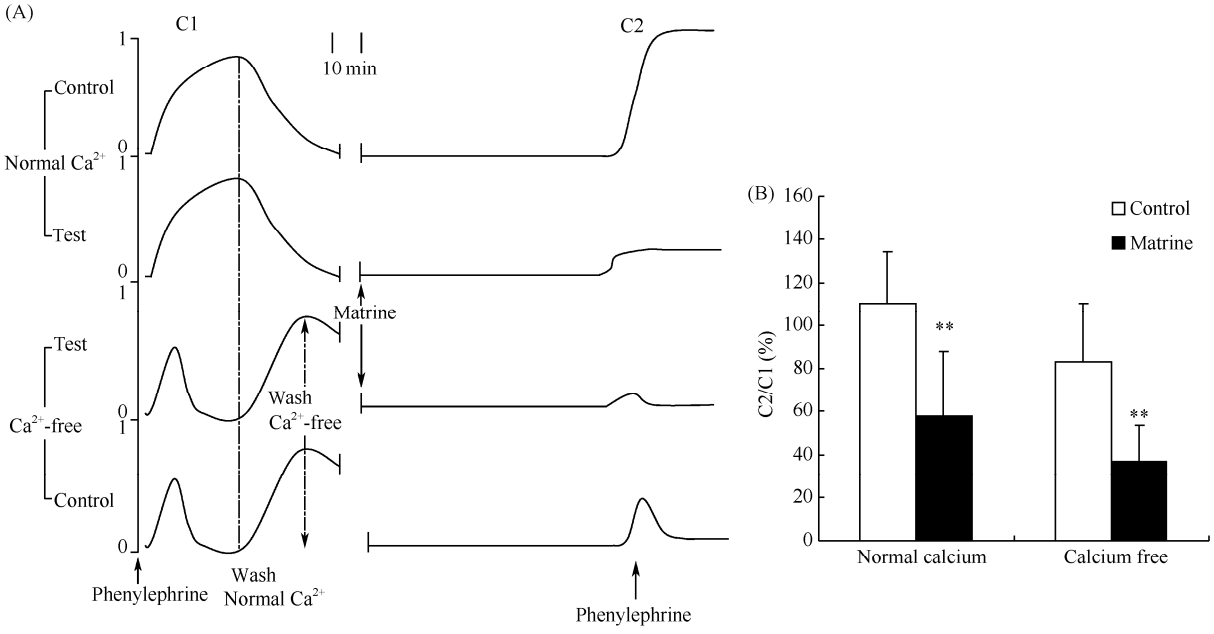

FIG. 4. Effect of matrine on phenylephrine-induced aortic smooth muscle contractions in experimental protocol (A) and in normal calcium and calcium-free Krebs' solutions (B). Vertical bars represent $\bar{x} \pm s$, $^{**}P<0.01$ *vs* control.

chloride and phenylephrine is different. Since potassium chloride-induced contraction results from the influx of Ca^{2+} on membrane, the smooth muscle will be depolarized in high external K^+, thereby opening voltage-dependent Ca^{2+} channels, with the potassium equilibrium potential shifted and the influx of calcium leading contractions[17-19]. The increased vascular tone caused by addition of phenylephrine to guinea-pig aortic rings is due to the activation of α_1-adrenoceptors[17,20]. Since matrine has been found to have direct and selective antagonizing effects on α-adrenoceptors, we examined phenylephrine-induced contractions of smooth muscle of aortic rings.

Calcium signaling is an important factor for vascular tone and contractility[21-22]. It has been reported that phenylephrine-induced aortic smooth muscle contraction is due to the activation of α_1-adrenoceptors leading to intracellular calcium release via the activation of inositol phosphate cascade and extracellular calcium influx via the receptor-operated Ca^{2+} channels, and that contractions to phenylephrine in a normal calcium bathing medium are dependent upon intracellular and extracellular calcium mobilization, whereas, in calcium free conditions, the response to phenylephrine is attributed to intracellular calcium[16-17]. The important finding of the present study is that matrine significantly decreases phenylephrine-induced contractions in both normal and Ca^{2+}-free Krebs' solutions, suggesting that it inhibits phenylephrine-induced contractions by interfering with both the release of intracellular Ca^{2+} and the influx of extracellular Ca^{2+}.

In this study, we used different inhibitors to determine the mechanism of matrine's relaxation of smooth muscle or aortic rings in guinea pigs.

The cardiovascular system is widely innervated by sympathetic sensory nerves and their receptors. Stimulation of α_1 receptors characteristically produces vasoconstriction and increases peripheral resistance and blood pressure[23]. Conversely, stimulation of β_2 receptors characteristically produces vasodilatation[24]. In order to test the contribution of β_2-adrenoceptors to the concentration-dependent matrine-induced vasorelaxation of the guinea-pig aortic smooth muscles, propranolol, a β-adrenergic antagonist, has been used[24-26]. In the present study, propranolol did not inhibit the relaxation of guinea-pig aortic smooth muscles, demonstrating that β_2-adrenoceptor mediated vaso-relaxation was not involved in the pathway.

Activation of K^+ channels plays an essential role in the regulation of vascular smooth muscles[15,17]. Opening of K^+ channels leads to dilation of vascular smooth muscles by hyperpolarizing the membrane[15]. In the present study, glibenclamide and tetraethylammonium were used respectively to analyze whether K^+ channels mediate relaxation of matrine-evoked guinea-pig aortic smooth muscle. Glibenclamide is a highly selective inhibitor of ATP-sensitive K^+ channels[27-28]. In our study, glibenclamide pretreatment did not inhibit the relaxant effects of matrine on guinea-pig aortic smooth muscles. As a non-selective K^+ channel inhibitor, tetraethylammonium can block most K^+ channels[27]. The concentration of tetraethylammonium used in the present study was sufficient to block K^+ channels, which is consistent with the reported findings[25], but actually the relaxation of aortic smooth muscles induced by matrine was not affected at such a concentration, which suggested that K^+ channels might not be involved in matrine-induced relaxation of the aortic muscle of guinea pigs.

In conclusion, matrine can inhibit phenylephrine-induced contractions by interfering with the release of intracellular Ca^{2+} and the influx of extracellular Ca^{2+} by blocking α_1 receptor.

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