Matrine protects homocysteine-induced atrial dysfunction in rats

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As one main alkaloid isolated from the seed or leaves of Chinese herb *Sophora alopecuroides* L. matrine has very strong therapeutic actions on stroke, heart infarction, hepatitis, pain, etc. In this study, we want to observe the preventive effects of matrine on systolic cardiac dysfunction induced by homocysteine. Wistar rat right atria were acutely isolated and the contraction of rat atria was determined by inductive force transducers. Confocal laser scanning microscopy was used to measure \([Ca^{2+}]_i\) in acutely isolated atrial myocytes. The results showed that matrine 10, 30, and 100 µM can effectively attenuated the decrease of the contractile force of rat atria in the presence of homocysteine 5 mM. Moreover, the effects of homocysteine 5 mM on +dT/dt and −dT/dt of rat atria also can be reversed by matrine 10, 30, and 100 µM. Further study uncovered that matrine restored the reduction of \([Ca^{2+}]_i\) caused by homocysteine 5 mM. These suggested that matrine can prevent homocysteine-induced atrial systolic dysfunction by regulating \([Ca^{2+}]_i\).

Key words: Matrine, inotropic effects, \([Ca^{2+}]_i\).

INTRODUCTION

*Sophora alopecuroides* L. is one kind of Chinese traditional herbs and has been used in Chinese Medicine to treat a variety of diseases for several hundred years. As one main active compound, matrine has more strong therapeutic effects on the disorders of brain, heart, liver, infection, and so on (Wei et al., 1985). It was demonstrated that both acute and chronic administration of matrine can improve cardiac functions and inhibit isoproterenol-induced ischemic injury via its antioxidant activity, but not affect heart rate and blood pressure (Li et al., 2010). Cardiac hypertrophy in cultured ventricular myocytes induced by endothelin-1 was markedly inhibited by matrine pretreatment via affecting the expression of β-MHC gene (Ruan et al., 2002). Matrine also played an antiarrhythmic role in patients with coronary heart diseases (Zhang et al., 2005), which possibly was associated with the prolongation of action potential by inhibiting inward rectifier potassium currents and increasing rapid delayed rectifier potassium currents (Zhou et al., 2007). It was also reported that matrine can inhibit aldosterone-induced the proliferation of rat cardiac fibroblasts in a dose dependent manner via influencing cell cycle (Hu et al., 2004). Though plenty of evidence confirmed that matrine exhibited effective therapeutic effects on ventricular myocardium, the information about the protective of matrine on the atrial dysfunction is in the lack. Homocysteine is an important risk factor for atrial remodeling (Cai et al., 2007a).

It was previously reported that elevated homocysteine level can induce the abnormalities of atrial systolic function and electrophysiology (Cai et al., 2007b, 2009; Joseph et al., 2004; Pacher et al., 1999). Whether matrine can exert preventive actions on homocysteine-induced contractile dysfunction is still unclear. So, the present study was designed to study the effect of matrine...
on homocysteine-induced the abnormalities of atrial functions.

MATERIALS AND METHODS

Animals

Male Wistar adult rats (230 to 270 g, 3.5 months old) were obtained from Experimental Animal Center of the Second Affiliated Hospital of Harbin Medical University, Harbin, China. Wistar rats were housed in stainless steel rust-free cages and had free access to food and distilled water (room temperature at 23 ± 1°C, humidity of 55 ± 5%, and 12 h dark/light cycle).

Reagent

Matrine was obtained from Kangjiu Chemical Co., China and dissolved in phosphate buffered solution. Flu-3/AM was purchased from Molecular probe. Homocysteine was obtained from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade. Deionized water was used throughout the experiments. Krebs-Henseleit buffer and Tyrode’s buffer solutions were freshly prepared from Molecular probe. Homocysteine was obtained from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade. Deionized water was used throughout the experiments. Krebs-Henseleit buffer and Tyrode’s buffer solutions were freshly prepared daily. The composition of the two buffer solutions were as follows: Krebs-Henseleit buffer (119.8 mM NaCl; 25 mM NaHCO3; 4.5 mM KCl; 1.2 mM MgSO4·7H2O; 1.35 mM CaCl2; 2H2O; 1.2 mM KH2PO4; 10 mM glucose; pH 7.35~7.45), and the Tyrode buffer (137 mM NaCl; 5.4 mM KCl; 1 mM MgCl2; 2 mM CaCl2; 10 mM glucose; 10 mM HEPES; pH 7.35~7.45).

Rat right atria preparation

The hearts were rapidly removed from rats after cervical dislocation and placed in Krebs-Henseleit buffer saturated with 95%O2 and 5%H2O2. The right atrium muscles were excised from isolated hearts, and then suspended in the 10 ml organ bath full of Krebs-Henseleit solution gassed with 95%O2 + 5%H2O2 at 37°C. Atrium muscles were stretched to the length of maximal force of contraction. The force of contractility (FOC) was measured with inductive force transducers (JZ101, Shanghai Sauter Bio-Technology Co., Ltd.) and analyzed by a BL-420E+ biological signal acquisition system (Chengdu Thai Union Electronics Co., Ltd.). The contractile force before (C0) and after (C1) drug administration were both recorded. The change of contraction (+C) was represented with the ratio of (C0-C1)/C0. All experimental procedures were approved by the University Committee on Animal Care and Supply of Harbin Medical University.

Measurement of contractile force

Contraction of rat atria was measured with inductive force transducers (JZ101, Shanghai Sauter Bio-Technology Co., Ltd.) and displayed on a computer screen using BL-420E + biological signal acquisition system (Chengdu Thai Union Electronics Co., Ltd.). Atria were allowed to equilibrate for 20 min before drug challenge. All experimental procedures were approved by the university committee on Animal Care and supply of Harbin Medical University.

Atrial myocytes isolation

Atrial myocytes were isolated enzymatically as previously described with some modification (Cai et al., 2009). Wistar rat hearts were quickly taken out after anaesthetized with pentobarbital (45 mg/kg, i.p), and then rapidly washed in cool, and oxygenated Tyrode buffer solution. After that, the washed hearts were mounted to a Langendorff perfusion apparatus and perfused with standard Tyrode’s solution for 5 min, and then switched to Ca2+-free Tyrode’s solution until it stopped beating, followed by perfusion with the same solution containing collagenase II (7.0 mg/50 ml) and bovine serum albumin (BSA). The right atrial tissue was shaved and minced in the storage solution and filtered. The freshly isolated atrial myocytes were gently centrifugated and resuspended in Ca2+-free Tyrode’s solution. Only single rod-shaped, Ca2+ tolerant, and quiescent cell with clear cross-striation was selected for the measurement of [Ca2+].

Measurement of [Ca2+]

Intracellular free calcium ([Ca2+]) level was measured using the fluorescent Ca2+ indicator Fluo-3/AM. In brief, the acutely isolated atrial cardiomyocytes on glass coverslips were washed in Tyrode’s buffer, and then loaded with 10 µM Fluo-3/AM (Eugene, OR, USA) containing 0.03% Pluronic F-127 for 35 min at 37°C and washed three times with Tyrode’s buffer to remove any extracellular dye. Fluorescent change of Fluo-3/AM loaded cells was detected by Confocal laser scanning microscope (Fluoview-FV300, Olympus, Japan) with 488 nm for excitation from an argon ion laser and 530 nm for emission and inverted microscope with 20×-objective. The fluorescent intensities before (FI0) and after (FI) drug administration were both recorded. Qualitative changes in [Ca2+], were inferred from the ratio of FI/FI0.

Statistical analysis

All data are represented as mean values ±SD. Student’s t test or one-way analysis of variance followed by Scheffe’s method for multiple comparisons was used. The criterion for statistical significance was that P values should be less than 0.05.

RESULTS

Initial experiments examined the effects of homocysteine on the force of contractility in rat atria. As shown in (Figure 1A), homocysteine 5 mM elicited a negative inotropic effect on rat atria (n = 5 independent experiments, p <0.05 compared to control atria). (Figure 1B) showed that pretreatment with matrine 10, 30 and 100 µM can markedly attenuate the negative inotropic effects of homocysteine on right atria (n = 6, 7 and 7 independent experiments for matrine 10, 20 and 30 µM, respectively, p <0.05 compared to homocysteine-treated atria). Moreover, matrine also can reverse the decreased maximum rate of tension increase (+dT/dt) and decline (−dT/dt) in the present of homocysteine 5 mM (Figures 1C and 1D).

Figure 2A demonstrated that homocysteine significantly inhibited the KCl-induced elevation of [Ca2+]i in isolated atrial myocytes (n = 30 cells for homocysteine treatment, p <0.05 compared to KCl-treated cells). Pretreatment with matrine 10, 30 and 100 µM can effectively prevent the decrease of [Ca2+]i induced by homocysteine (n = 30 cells for each concentration of matrine, p <0.05 compared to
Figure 1. Matrine prevented contraction dysfunction induced by homocysteine (HCY) in rat atria. Superimposed tension recordings from rat right atria were shown in Figure 1A. Matrine can prevent the reduction of contractile force (Figure 1B), maximal rate of tension increase (+dt/dt) (Figure 1C) and maximal rate of tension decline (-dT/dt) (Figure 1D). * p<0.05 vs control atria, # p<0.05 vs homocysteine-treated atria.

DISCUSSION

*S. alopecuroides* is a common traditional Chinese medicine and widely used in China. Alkaloids extracted from *S. alopecuroides* such as matrine and oxymatrine have shown multiple biological activities (Wei et al., 1985). Recently, increasing evidence showed that matrine evoked a strong antiarrhythmic action on animals and patient with coronary through influencing several potassium channels currents (Zhang et al., 2005; Zhou et al., 2007). Matrine also can attenuate cardiac hypertrophy and the increased expression of β-MHC gene in cultured ventricular myocytes induced by endothelin-1 (Ruan et al., 2002). Moreover, aldosterone-induced cardiac fibrosis may be effective protected by matrine via inhibiting proliferation of cardiac fibroblast (Hu et al., 2004). Ai et al. (2001) found that matrine between 1 to 100 µM can elevate intracellular [Ca$^{2+}$], in the presence of extracellular calcium and enhance KCl-induced the increase of [Ca$^{2+}$]. It was further revealed that matrine exhibited positive inotropic effects on electrically driven guinea pig papillary muscles via enhancing the KCl-induced elevations of [Ca$^{2+}$] and increasing cardiac calcium currents in a dose-dependent manner (Zhou et al., 2008). In aortic rings, matrine can relax the phenylephrine-induced vascular contractions but not the potassium chloride-induced contraction (Zheng et al., 2009). Its mechanism was associated with release of intracellular Ca$^{2+}$ and the influx of extracellular Ca$^{2+}$. However, the information about the protective effects of matrine on cardiac disorders in atria is still limited.

Increased plasma homocysteine level is one novel important independent risk factor for cardiovascular and...
cerebrovascular diseases (Joseph et al., 2004; Pacher et al., 1999).

Our previous studies uncovered that homocysteine decreased transient outward potassium currents and ultra-rapidly delayed rectifier potassium currents, increased sodium currents and inward rectifier potassium currents in human atrial myocytes (Cai et al., 2007b, 2009), which suggested homocysteine-induced electrophysiological alteration contributed to hyperhomocysteinemia-related atrial abnormalities. Homocysteine also exerted a regulatory action on the depolarization and repolarization of action potential in atria (Pacher et al., 1999).

In clinics, patients with hyperhomocysteinemia were often accompanied by atrial dysfunctions (Cupini and De Simone, 2003). Accordingly, it is very necessary to look for new drugs to treat homocysteine-induced atrial dysfunctions.

We found that matrine pretreatment can protect rat right atria from homocysteine-induced dysfunction in atrial contraction. Homocysteine 5 mM produced a significant decrease of contractile force of rat atria, indicating homocysteine induced atrial dysfunction. Matrine 10, 30, and 100 µM can effectively reverse the reduction of atrial contraction in the present of homocysteine. Meanwhile, the decrease of +dT/dt and -dT/dt in the present of homocysteine was also markedly attenuated by matrine pretreatment. These suggested matrine can treat homocysteine-correlated atrial disorders.

It is well known that \([\text{Ca}^{2+}]\), played an important regulatory role in the contraction and dilation of muscles (Asadollahi et al., 2010). Then augment of \([\text{Ca}^{2+}]\), can enhance the contraction of rat atria. Conversely, the reduction of \([\text{Ca}^{2+}]\), will cause the dysfunction of rat atria contraction. In this study, we found that elevation of \([\text{Ca}^{2+}]\), induced by KCl was strongly inhibited by homocysteine. Matrine pretreatment can ameliorate the inhibition of \([\text{Ca}^{2+}]\), in the present of homocysteine, indicating the regulation of \([\text{Ca}^{2+}]\), by matrine contributed to its therapeutic effects of homocysteine-related atrial dysfunction.

Conclusion

Matrine can reverse the atrial contraction dysfunction induced by homocysteine, which was associated with the regulation of \([\text{Ca}^{2+}]\), by matrine. The study provided one insight for the treatment of homocysteine-related atrial disorders.

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