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The alkaloid matrine of the root of Sophora flavescens prevents arrhythmogenic effect of ouabain

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ABSTRACT

Matrine, a alkaloid of the root of Sophora flavescens, has multiple protective effects on the cardiovascular system including cardiac arrhythmias. However, the molecular and ionic mechanisms of matrine have not been well investigated. Our study aimed at to shed a light on the issue to investigate the antiarrhythmic effects of matrine by using ouabain to construct an arrhythmic model of cardiomyocytes. In this experiment, matrine significantly and dose-dependently increased the doses of ouabain required to induce cardiac arrhythmias and decreased the duration of arrhythmias in guinea pigs. In cardiomyocytes of guinea pigs, ouabain 10 μM prolonged action potential duration by 80% ($p < 0.05$) and increased L-type Ca^{2+} currents and Ca^{2+} transients induced by KCl ($p < 0.05$). Matrine 100 μM shortened the prolongation of APD and prevented the increase of L-type Ca^{2+} currents and Ca^{2+} transients induced by ouabain. Taken together, these findings provide the first evidence that matrine possessed arrhythmogenic effect of ouabain by inhibiting of L-type Ca^{2+} currents and Ca^{2+} overload in guinea pigs.

Introduction

Matrine is an alkaloid (Fig. 1) isolated from Sophora flavescens Ait (SF) and has been identified as the bioactive component contributing to a variety of pharmacological effects such as hepatitis B and C (Lao 2005; Zhang and Huang 2004), cancers (Long et al. 2004), and cardiac diseases (Li et al. 2009). In clinics, matrine is currently used to treat cardiac arrhythmias, especially premature ventricular beats (Zhang et al. 2005). Dai et al. (1986) reported that the effects of an ethanol extract of the plant Sophora flavescens Ait possessed antiarrhythmic activity. Based on our previous research, we found that matrine possessed antiarrhythmic effect in experimental arrhythmic models induced by coronary artery ligation and electric stimulation in rats and rabbits (Xu et al. 2004). Matrine exerts its anti-AF effects by down-regulation of I_{KM3} density and up-regulation of $I_{Ca\text{-}L}$ density (Zhou et al. 2012). In addition, matrine enhanced $[Ca^{2+}]_i$ by stimulating $I_{Ca\text{-}L}$ and exerted positive inotropic effects in guinea pigs papillary muscles (Zhou et al. 2008).

It was well known that ouabain is a widely used agent to screen and evaluate antiarrhythmic drugs all over the world. It is used to develop stable and ideal animal models for arrhythmias. The classical mechanism of ouabain was related with its binding to and inhibition of the plasma membrane Na^+/K^+-ATPase (sodium pump) and induced an increase in intracellular Ca^{2+} concentration leading to extrasystoles and ventricular arrhythmias (Nesher et al. 2010; Lingrel 2010; Sapia et al. 2010; Zhang et al. 2010). In our experiment, we aimed to construct the arrhythmic model in myocytes and mimic ionic changes in occurrence of arrhythmias by using ouabain. Then we observed the effects of matrine on ionic currents pretreated with ouabain. Matrine possess well antiarrhythmic effect, but little is known about the ionic mechanisms of matrine on arrhythmias induced by ouabain in guinea pigs. So our study investigated the effects of matrine on arrhythmias in guinea pigs and ionic changes in cardiac myocytes induced by ouabain to find out its antiarrhythmic mechanism.

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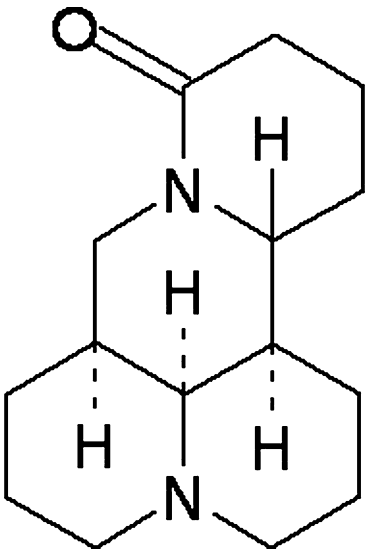

Fig. 1. Chemical structure of matrine (C15H24N2O, molecular weight = 248.36).

Materials and methods

Animals

Guinea pigs (300–400 g, the Animal Center of Harbin Medical University) were housed at 20 ± 3 °C with 55 ± 10% humidity, 12 h light/dark cycle and free access to species-specific food and tap water. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Studies were approved by the Institute Committee of the Animal Care of Harbin Medical University.

Chemicals

Matrine (≥98%, purity by HPLC) was purchased from Xian Botany Garden (Shanxi, China). Matrine stock solution was prepared in di-distilled water (ddH_2O) at 10 mM. 4-(2-hydroxyehtyl) piperazine-1-ethanesulfonic acid (HEPES), Na_2-ATP, K asparate, collagenase (II-type) were purchased from Sigma (St Louis, MO, U. S. A.).

Ouabain induced arrhythmia

Fourty guinea pigs were randomly assigned into four groups that were matrine 5 mg/kg, matrine 15 mg/kg, matrine 45 mg/kg, and saline control group. They were anesthetized with pentobarbital (40 mg/kg, iv). The standard limb II ECG was continuously recorded by a recorder (BL420, TaiMeng, China). Ten minutes after the administration of matrine (iv), ouabain (18 μg/kg) was pumped at a speed of 0.5 ml/min.

Criteria of arrhythmias and death

The survival rate and the incidence of arrhythmias were registered and evaluated in accordance with the Lambeth Conventions (Walker et al. 1988). That is, ectopic ventricular activity is categorized as a single premature ventricular contraction (PVC), ventricular tachycardia (VT, 4 or more consecutive PVC) or ventricular fibrillation (VF, inability to distinguish individual QRS complexes or to measure the rate). Complex forms (e.g., bigeminy) were included in the count of PVC and were not analyzed separately. In drug induced arrhythmic experiments, we record the dosages of ouabain which caused PVC, VT, and VF.

Electrophysiological recordings

Cardiac myocytes were isolated from the ventricles of Langendorff-perfused guinea pig hearts. Currents were recorded using standard whole-cell patch clamp techniques and voltage-clamp experiments were performed with an Axopatch 700A amplifier (Axon Instruments). The recording electrodes (Borosilicate glass, Sutter) were pulled (P-87, Sutter Instruments) and polished (F-83, Narishige) down to 2–3 MΩ when filled with pipette solution (in mM: NaCl 10, KCl 50, K_2SO_4 50, and $MgCl_2$ 5.0, pH adjusted with NaOH to 7.35). The mean capacitance of cells included in this experiment was (128 ± 11) pF (n = 24). After the formation of the gigaohm-seal, the capacitance was electronically compensated by 80%. Then the cell membrane under the pipette tip was then ruptured by a brief increase in suction, forming the whole-cell recording configuration. The series resistance was electrically compensated by 70–80%. All cells were recorded at room temperature (22–23 °C).

For measurement of inward Ca^{2+} currents, the bath Tyrode's solution was contained in mM: 136 Tris–HCl, 5.4 CsCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 0.33 NaH_2PO_4, 10 glucose, and 10 HEPES. pH was adjusted to 7.4 with Tris–OH. The pipette solution was contained in mM: CsOH 125; Aspartic acid 35; Tetraethylammonium chloride 30; HEPES 11; Mg-ATP 5.0; EGTA 10; Phosphocreatine 3.6, pH was adjusted with 1 N CsOH to 7.30.

Current amplitude data of each cell were normalized to its cell capacitance (current density, pA/pF). Current–voltage relationship (I–V curve) was presented by the currents normalized by the peak currents. Voltage-dependent activation and steady-state inactivation profiles were calculated by Boltzmann fitting function. The activation curves were obtained by fitting the data points to a Boltzmann equation of the form $G/G_{max} = 1/\{1 + \exp[(V_h - V_m)/k]\}$ where G_{max} is the maximum conductance of the voltage-gated Ca^{2+} channels, V_{rev} is the extrapolated reversal potential of I, $V^{½}$ is the potential for half-maximal conductance, and k is the slope.

The curve for voltage dependence of steady state inactivation was obtained by fitting the data to a Boltzmann distribution of the form. $I/I_{max} = I/\{I + \exp[(V_m - V_h)/k]\}$ where I gives the current amplitude and Imax its maximum, V_m the potential of prepulse, V_h the half-maximal inactivation potential, and k the slope factor.

Measurement of $[Ca^{2+}]_i$

Isolated ventricular myocytes were adhered to the cover-slip of the chamber and cells were rinsed once with standard Tyrode's solution and incubated with a working solution containing Fluo-3/AM (20 μM) and Pluronic F-127 (0.03%) at 37 °C for 45 min. After loading, cells were washed once with standard Tyrode's solution to remove the extracellular Fluo-3/AM. Fluorescent changes of Fluo-3/AM-loaded cells were detected by laser scanning confocal microscope with 488 nm for excitation from an argon ion laser and 530 nm for emission and inverted microscope with 20 × objective. Drugs were added between 3rd and 4th scan. The fluorescent intensities and images were stored in disks. The fluorescent intensities before (FI_0) and after (FI) drug administration were both recorded. The change of $[Ca^{2+}]_i$ was represented as the ratio of FI/FI_0.

Statistics

Results are expressed as mean ± S.E.M. and statistical comparisons (performed using ANOVA followed by Dunnett's method)