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Effects of matrine, artemisinin, and tetrandrine on cytosolic $[Ca^{2+}]_i$ in guinea pig ventricular myocytes[1]

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KEY WORDS matrine; artemisinin; tetrandrine; calcium; myocardium; confocal microscopy

ABSTRACT

AIM: To compare the effects of matrine, artemisinin, and tetrandrine on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in guinea pig ventricular myocytes. **METHODS**: A single ventricular myocyte was loaded with Fluo 3-acetoxymethyl (Fluo 3-AM). $[Ca^{2+}]_i$ was recorded by laser scanning confocal microscope and represented by fluorescence intensity (FI). **RESULTS**: 1) KCl 60 $mmol \cdot L^{-1}$ elevated the FI from 299 ± 19 to 1389 ± 325 ($P < 0.01$) in the presence of extracellular Ca^{2+} 1.8 $mmol \cdot L^{-1}$. 2) Both matrine and artemisinin at the concentration of 100 $\mu mol \cdot L^{-1}$ could enhance the increase of FI by KCl 60 $mmol \cdot L^{-1}$. The FI values reached 1495 ± 320 and 1646 ± 308 from 301 ± 14 and 299 ± 16 ($P < 0.01$), respectively. 3) Both tetrandrine 1, 10, and 100 $\mu mol \cdot L^{-1}$ and verapamil 10 $\mu mol \cdot L^{-1}$ inhibited the influx of extracellular Ca^{2+} induced by KCl 60 $mmol \cdot L^{-1}$. 4) Matrine 1, 10, and 100 $\mu mol \cdot L^{-1}$ could elevate the FI in the presence of extracellular Ca^{2+}. The FI values reached 441 ± 96, 504 ± 112, and 643 ± 126 from 303 ± 27, 300 ± 32, and 296 ± 19 ($P < 0.05$), respectively. 5) Tetrandrine 1 and 10 $\mu mol \cdot L^{-1}$ could apparently inhibited Ca^{2+} release from intracellular calcium stores induced by caffeine 20 $mmol \cdot L^{-1}$ ($P < 0.05$). **CONCLUSION**: Effects of matrine, artemisinin, and tetrandrine on $[Ca^{2+}]_i$ in ventricular myocytes were different. Both artemisinin and matrine could enhance Ca^{2+} entry induced by KCl, while tetrandrine, as verapamil did, inhibited this kind of Ca^{2+} entry. Matrine itself could produce Ca^{2+} entry.

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INTRODUCTION

Matrine, extracted from the dried root of *Sophora flavescens*, showed the antiarrythmic action in animal models which induced by aconitine, chloroform, adrenaline, and coronary artery ligation[1,2]. However, the mechanism of matrine is not clear.

Artemisinin, an antimalarial drug, extracted from the dried portion of *Artemisia annua* L. In recent years, it has been found that artemisinin has the pharmacological effects of antiarrhythmia[3]. The study by whole cell patch-clamp technique indicated that artemisinin could block the two components (I_{Kr} and I_{Ks}) of delayed outward rectified K^+ current in ventricular cells isolated from guinea pig[4]. But the effects of artemisinin on intracellular calcium ions have not been reported.

Tetrandrine, extracted from *Stephania tetrandra* S Moore, showed a noncompetitive calcium antagonism like verapamil on isolated cardiomyocytes in rat. Tetrandrine inhibited both L-type and T-type voltage-dependent Ca^{2+} channel[5]. At high concentrations, tetrandrine reduced the magnitude of caffeine-induced $[Ca^{2+}]_i$ transient[6,7].

Matrine, artemisinin, and tetrandrine all have the action of antiarrythmia, however, we still do not know if they have the same effects on $[Ca^{2+}]_i$. The present study was to compare the effects of matrine, artemisinin, and tetrandrine on $[Ca^{2+}]_i$ in ventricular myocytes isolated from guinea pigs.

MATERIALS AND METHODS

Animals and agents Guinea pigs were provided by Animal Center of Harbin Medical University, weighing (320 ± 20) g. Fluo 3-acetoxymethyl (Fluo 3-AM) ester (Molecular Probes Eugere OR, USA) was dissolved in Me_2SO and the final concentration was 1 $g \cdot L^{-1}$. The solution was stored at -20 ℃ in dark. Collagenase Ⅱ was obtained from CLS Washington Biochemical (USA). Pluronic F-127, 4-(2-Hydroxyethyl)-1-piperazineethane-

sulfonic acid (HEPES), egtazic acid, taurine, glutamic acid, and Me_2SO were purchased from Sigma (USA). Matrine, tetrandrine, and artemisinin were provided by Pharmaceutical Institute of the Second Affiliated Hospital of Harbin Medical University (China). The purity of these drugs was 98 %.

Isolation of single ventricular myocyte A single ventricular cell was isolated from guinea pig heart as described previously[8]. Briefly, guinea pig was fainted by a cervical dislocation, then the heart was quickly removed and cannulated on a Langendorff apparatus and perfused with Tyrode's solution (mmol·L^{-1}: NaCl 135, KCl 4, $CaCl_2$ 1, HEPES 10, and Glucose 10, adjusted pH to 7.35 – 7.45 with HCl at 36 ℃) for 2 – 3 min, to eliminate remained blood. After the heart was perfused with Ca^{2+}-free Tyrode's solution for 8 – 10 min, it was enzymatically digested for a period of 15 – 20 min with a solution containing collagenase Ⅱ 0.16 g·L^{-1}. Left ventricular tissue was then excised from the softened heart, placed in a Kraftbruhe (KB) solution (mmol·L^{-1}: Glutamic acid 70, Taurin 15, KCl 30, $MgCl_2$ 0.5, HEPES 10, KH_2PO_4 10, Glucose 10, and egtazic acid 0.5, adjusted pH to 7.30 – 7.40 with 5 mol·L^{-1} of KOH), and gently blowed. The myocytes were incubated in the KB solution at 22 – 25 ℃ for 1 h.

Flou 3-AM loading Isolated single cardiomyocyte was taken from KB solution by centrifugation at 14.8 × *g* for 1 min, and loaded with Flou 3-AM 1 % working solution containing 0.01 % pluronic F-127 at 37 ℃ for 1 h. The cells were then washed with Ca^{2+}-free PBS by three times to remove the extracellular Flou 3-AM[9].

Laser scanning The loaded cells with bar-shaped and clear striates were mounted in a chamber. Fluo-3 fluorescence was detected with an INSIGHT PLUS laser scanning confocal microscope system equipped with an Olympus IMT-Ⅱ inverted microscope. An argon laser was used to excite Fluo-3 at 488 nm and emit at 530 nm. $[Ca^{2+}]_i$ changes were represented with fluoresence intensity (FI).

Time series of optical sections through a cell was obtained with a XY-step. Total 25 images were scanned with each experiment and the data were stored in disks.

Statistic analysis Results were presented as $\bar{x} \pm s$. Student's *t*-test was used in statistical comparison.

RESULTS

Effect of matrine on $[Ca^{2+}]_i$ KCl 60 mmol·L^{-1} increased the $[Ca^{2+}]_i$ in single isolated cardiomyocyte (Tab 1). After the preincubation with matrine 1, 10, and 100 μmol·L^{-1}, the increase of $[Ca^{2+}]_i$ induced by KCl was not decreased, but was higher than that of KCl alone ($P < 0.05$). Matrine increased $[Ca^{2+}]_i$ in normal extracellular Ca^{2+} solution at the rest level in a concentration-dependent manner ($P < 0.05$, Tab 2), while it did not affect $[Ca^{2+}]_i$ in Ca^{2+}-free solution ($P > 0.05$, Tab 3).

Tab 1. Effects of matrine, artemisinin, tetrandrine, and verapamil on $[Ca^{2+}]_i$ elevation induced by KCl 60 mmol·L^{-1} and caffeine 20 mmol·L^{-1} in single ventricular myocyte from guinea pigs. $[Ca^{2+}]_i$ changes were represented by fluoresence intensity (FI). n = 18 – 22 cardiomyocytes from 6 – 8 guinea pigs. $\bar{x} \pm s$. $^{c}P < 0.01$ *vs* control. $^{d}P > 0.05$, $^{f}P < 0.01$ *vs* KCl. $^{i}P < 0.01$ *vs* caffeine.

Drug/μmol·L^{-1}	n	$[Ca^{2+}]_i$/FI	
		Control	Peak value
KCl 60 mmol·L^{-1}	22	299 ± 14	1389 ± 325^{c}
Matrine			
1	20	301 ± 18	1325 ± 286^{d}
10	20	297 ± 23	1353 ± 318^{d}
100	19	301 ± 14	1495 ± 320^{f}
Artemisinin			
1	18	298 ± 12	1430 ± 395^{d}
10	18	300 ± 17	1589 ± 389^{f}
100	19	299 ± 14	1646 ± 365^{f}
Tetrandrine			
1	20	302 ± 22	602 ± 96^{f}
10	18	301 ± 9	437 ± 105^{f}
100	18	304 ± 15	455 ± 84^{f}
verapamil			
10	18	306 ± 6	466 ± 72^{f}
Caffeine 20 mmol·L^{-1}	18	302 ± 13	1105 ± 323^{c}
Tetrandrine			
1	22	300 ± 4	802 ± 284^{i}
10	22	301 ± 12	514 ± 151^{i}

Effect of artemisinin on $[Ca^{2+}]_i$ Unlike matrine, artemisinin did not affect the FI value in this kind of cells ($P > 0.05$, Tab 2 and 3). In the presence of artemisinin 1, 10, and 100 μmol·L^{-1}, the increase of $[Ca^{2+}]_i$ induced by KCl 60 mmol·L^{-1} was further increased ($P < 0.01$, Tab 1 and Fig 1).

Effect of tetrandrine on $[Ca^{2+}]_i$ The increase of $[Ca^{2+}]_i$ induced by KCl 60 mmol·L^{-1} was inhibited by tetrandrine 1, 10, and 100 μmol·L^{-1}. The inhibitory effect of tetrandrine 10 μmol·L^{-1} was similar to that of verapamil 10 μmol·L^{-1} ($P < 0.05$, Tab 1). Caffeine

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Tab 2. Effects of matrine and artemisinin on $[Ca^{2+}]_i$ in normal extracellular solution. $[Ca^{2+}]_i$ changes were represented by fluoresence intensity (FI). $n = 18-19$ cardiomyocytes from 6-8 guinea pigs. $\bar{x} \pm s$. [a]$P > 0.05$, [b]$P < 0.05$, [c]$P < 0.01$ vs control.

Drug/μmol·L⁻¹	n	$[Ca^{2+}]_i$/FI Control	Peak value
Matrine			
1	18	303 ± 27	441 ± 27[b]
10	18	300 ± 32	504 ± 112[b]
100	19	297 ± 19	643 ± 126[c]
Artemisinin			
1	18	302 ± 8	311 ± 14[a]
10	18	298 ± 7	304 ± 6[a]
100	18	306 ± 18	316 ± 13[a]

Tab 3. Effects of matrine and artemisinin on $[Ca^{2+}]_i$ in Ca^{2+}-free extracellular solution. $[Ca^{2+}]_i$ changes were represented by fluoresence intensity (FI). $n = 18-20$ cardiomyocytes from 6-8 guinea pigs. $\bar{x} \pm s$. [a]$P > 0.05$ vs control.

Drug/μmol·L⁻¹	n	$[Ca^{2+}]_i$/FI Control	Peak value
Matrine			
1	18	298 ± 4	306 ± 6[a]
10	20	303 ± 6	304 ± 11[a]
100	20	297 ± 5	303 ± 10[a]
Artemisinin			
1	18	306 ± 8	310 ± 12[a]
10	18	302 ± 7	309 ± 10[a]
100	19	301 ± 5	312 ± 14[a]

20 mmol·L⁻¹ elevated $[Ca^{2+}]_i$, which was inhibited by tetrandrine 1 and 10 μmol·L⁻¹ in Ca^{2+}-free extracellular solution ($P < 0.01$, Tab 1 and Fig 1).

DISCUSSION

The results demonstrated that matrine could not inhibit voltage-dependent Ca^{2+} channel of ventricular myocyte, because $[Ca^{2+}]_i$ mobilizations by high K^+ depolarization were not affected by matrine. In the present experiment, matrine had no effect on calcium release from internal stores in the Ca^{2+}-free extracellular solution, but it could elevate $[Ca^{2+}]_i$ in the normal extracellular solution. Artemisinin had no effect on the $[Ca^{2+}]_i$ in normal

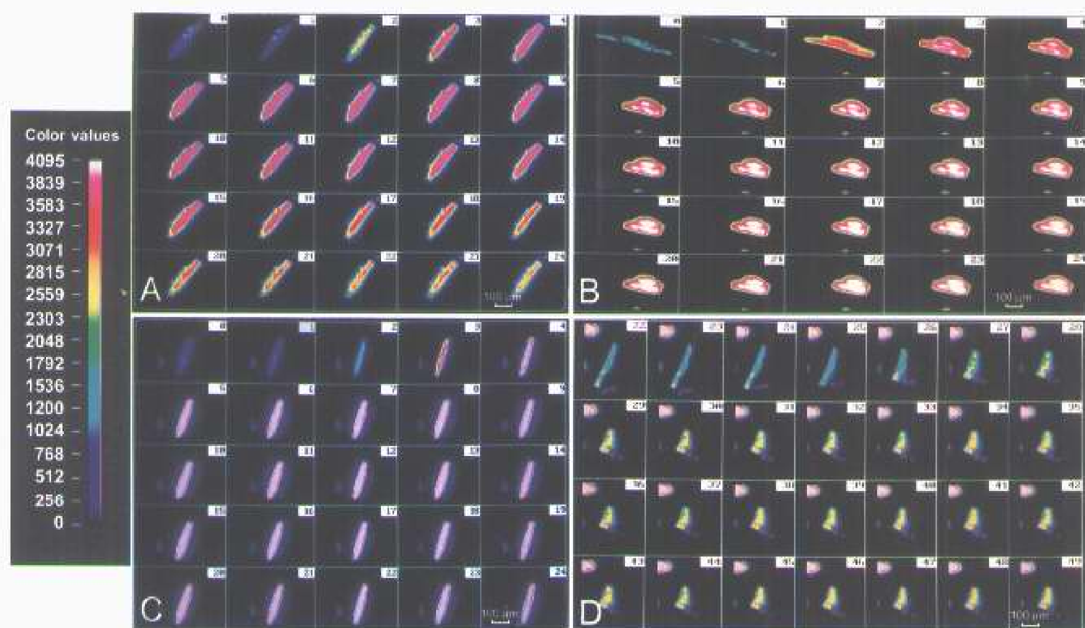

Fig 1. Image matrix of calcium fluorescent images. A) Fluorescent images induced by KCl 60 mmol·L⁻¹; B) Fluorescent images mobilization by caffeine 20 mmol·L⁻¹; C) Effect of artemisinin 100 μmol·L⁻¹ on $[Ca^{2+}]_i$ induced by KCl 60 mmol·L⁻¹; D) Effect of tetrandrine 100 μmol·L⁻¹ on $[Ca^{2+}]_i$ induced by caffeine 20 mmol·L⁻¹. Calcium fluorescence intensity (FI) was represented by various colors. ×40.

and Ca^{2+}-free extracellular solution, but it could enhance the action of KCl. It suggested that artemisinin might affect $[Ca^{2+}]_i$ through acting on voltage-dependent Ca^{2+} channel. Our study showed that the increase of $[Ca^{2+}]_i$ induced by KCl were transient, but the increase turned to be permanent after the cells were pretreated with artemisinin for 10 min.

In the present research, tetrandrine could inhibit voltage-dependent Ca^{2+} channel. This result was consistent with the previous report. In addition, our results also showed that tetrandrine could inhibit Ca^{2+} release from internal store since the increase of $[Ca^{2+}]_i$ induced by caffeine was inhibited by tetrandrine. But it could not inhibit the contraction induced by caffeine (Fig 1B and D).

It was concluded that the effects of matrine, artemisinin, and tetrandrine on cytosolic $[Ca^{2+}]_i$ in ventricular myocytes were different according to the results mentioned above. Artemisinin and tetrandrine could affect the voltage-dependant Ca^{2+} channel, but the mechanisms are different. Matrine had no effect on voltage-dependant calcium channel, but could affect the $[Ca^{2+}]_i$ itself. To further confirm whether matrine could promote Ca^{2+} influx from extracellular space and if artemisinin could affect the dynamics of calcium, calcium current recording by patch-clamp technique should be studied.

REFERENCES

1 Zhang BH, Wang NS, Li XJ, Kong XJ, Cai YL. Anti-arrhythmic effects of matrine. Acta Pharmacol Sin 1990; 11: 253-6.

2 Zhang MF, Shen YQ. Anti-arrhythmic effects of matrine type alkaloid. Chin Pharmacol Bull 1989; 5: 148-251.

3 Wang HZ, Yang BF, Luo DL, Zhang J, Liao SJ. The anti-arrhythmic effects of artemisinin on arrhythmias. Chin Pharmacol Bull 1998; 14.

4 Yang BF, Luo DL, Bao LH, Zhang YC, Wang HZ. Artemisinin blocks activating and slowly activating K^+ current in guinea pig ventricular myocytes. Acta Pharmacol Sin 1998; 19: 269-72.

5 Liu QY, Karpiski E, Parg PK. Tetrandrine inhibits both T and L calcium channel currents in ventricular cells. J Cardiovasc Pharmacol 1992; 20: 513-9.

6 Takemura H, Moto K, Ohshica. Tetrandrine as a calcium antagonist. Clin Exp Pharmacol Physiol 1996; 23: 751-3.

7 Wang HX, Kan CY, Wong TM. Tetrandrine inhibits electrically induced $[Ca^{2+}]_i$ transient in the isolated single rat cardiomyocyte. Eur J Pharmacol 1997; 14: 115-22.

8 Yang BF, Li YR, Xu CQ, Luo DL, Li BX, Wang HZ, *et al*. Mechanisms of artemisinin antiarrhythmic action. Chin J Pharmacol Toxicol 1999; 13: 169-75.

9 Dong DL, Sun JP, Luo DL, Chen QW, Wang F, He SZ, *et al*. Effect of berberine on the cytosolic calcium concentration in the single ventricullar cell of guinea pig. Chin J Pharmacol Toxicol 2000; 14: 128-30.

苦参碱、青蒿素和粉防己碱对豚鼠心室肌细胞胞浆钙的影响[1]

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关键词　苦参碱；青蒿素；粉防己碱；钙；心肌；共聚焦显微镜检查

目的：比较苦参碱、青蒿素和粉防己碱对分离的豚鼠心室肌细胞胞浆钙的影响．**方法**：用酶解法急性分离的豚鼠心室肌细胞先用 Fluo 3-AM 负载，然后用激光扫描共聚焦法检测单个豚鼠心室肌细胞胞浆钙的荧光强度．**结果**：(1) KCl 60 $mmol \cdot L^{-1}$可升高心室肌细胞胞浆钙荧光强度，其荧光强度从 299±19 升高到 1389±325 ($P<0.01$)．(2) 苦参碱和青蒿素在浓度为 100 $\mu mol \cdot L^{-1}$可使 KCl 60 $mmol \cdot L^{-1}$引起的外钙内流增多，其荧光强度分别从 301±14 和 299±16 升高到 1495±320 和 1646±308 ($P<0.05$)．(3) 粉防己碱 1、10 及 100 $\mu mol \cdot L^{-1}$和维拉帕米 10 $\mu mol \cdot L^{-1}$可抑制 KCl 引起的外钙内流．(4) 在正常细胞外液中，苦参碱 1、10 和 100 $\mu mol \cdot L^{-1}$可使细胞内荧光强度增强，其荧光强度分别从 303±27，300±32，296±19 上升到 441±96，504±112，643±126 ($P<0.05$)．(5) 在无钙细胞外液中，粉防己碱 1 和 10 $\mu mol \cdot L^{-1}$可明显抑制咖啡因 20 $mmol \cdot L^{-1}$引起的胞浆钙升高 ($P<0.05$)．**结论**：苦参碱、青蒿素和粉防己碱对豚鼠心室肌细胞胞浆钙的影响不同．青蒿素和苦参碱可加强电压依赖性钙内流，而粉防己碱与维拉帕米作用相似，抑制这种钙内流．苦参碱本身可以促进外钙内流．

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