Potential Antiarrhythmic Effect of Methyl 3,4,5-Trimethoxycinnamate, a Bioactive Substance from Roots of *Polygalae Radix*: Suppression of Triggered Activities in Rabbit Myocytes

Zhenghang Zhao,^{*,a,b} Minfeng Fang,^c Dandan Xiao,^a Mei Liu,^a Nadezhda Fefelova,^b Chen Huang,^a Wei-Jin Zang,^a and Lai-Hua Xie^b

^aDepartment of Pharmacology, School of Medicine, Xi'an Jiaotong University; Xi'an 710061, China; ^bDepartment of Cell Biology and Molecular Medicine, New Jersey Medical School, University of Medicine and Dentistry of New Jersey (UMDNJ); Newark, NJ 07101, U.S.A.; and ^cKey Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, College of Life Sciences, Northwest University; Xi'an 710069, China.

Received July 25, 2012; accepted November 13, 2012; advance publication released online November 29, 2012

3,4,5-Trimethoxycinnamic acid (TMCA), methyl 3,4,5-trimethoxycinnamate (M-TMCA) and *p*-methoxycinnamic acid (PMCA) have been identified as the major bioactive components in the serum collected from rats treated with oral administration of *Polygalae Radix* ("YuanZhi," the roots of *Polygala tenuifolia* WILLD.), a traditional Chinese medicine used to relieve insomnia, anxiety and heart palpitation. The present study was designed to investigate its direct electrophysiological effects on isolated ventricular myocytes from rabbits. Whole-cell configuration of the patch-clamp technique was used to measure action potential (AP) and membrane currents in single ventricular myocytes enzymatically isolated from adult rabbit hearts. Ca^{2+} transients were recorded in myocytes loaded with the Ca^{2+} indicator Fluo-4AM. Among three bioactive substances of *Polygala* metabolites, only M-TMCA (15–30 μM) significantly shortened action potential duration at 50% and 90% repolarization (APD_{50} and APD_{90}) in cardiomyocytes in a concentration-dependent and a reversible manner. M-TMCA also inhibited L-type calcium current ($I_{\text{Ca,L}}$), but showed effect on neither transient outward potassium current (I_{to}) nor steady-state potassium current ($I_{\text{K,ss}}$). Furthermore, M-TMCA abolished isoprenaline plus BayK8644-induced early afterdepolarizations (EADs) and suppressed delayed afterdepolarizations (DADs) and triggered activities (TAs). This potential anti-arrhythmic effects were likely attributed by the inhibition of $I_{\text{Ca,L}}$ and the suppression of intracellular Ca^{2+} transients, which consequently suppress the generation of transient inward current (I_{ti}). These findings suggest that M-TMCA may protect the heart from arrhythmias *via* its inhibitory effect on calcium channel.

Key words methyl 3,4,5-trimethoxycinnamate; L-type calcium current; cardiomyocyte; action potential; Ca^{2+} transient; *Polygalae Radix*

Polygalae Radix (Yuan Zhi, *Polygala tenuifolia* WILLD.) is a well known Chinese traditional medicine used as a sedative, expectorant and tonic agent. It has been widely used to treat insomnia, anxiety, restlessness and disorientation.^{1,2)} The studies on the chemical components of this crude drug have demonstrated the presence of polygalitol, *N*-acetyl-D-glucosamine, onjisaponins A–G,³⁾ various xanthenes,⁴⁾ tenuifoliosides A–P, 3,4,5-trimethoxycinnamic acid (TMCA), tenuifoliosides A–D and sucrose derivatives.⁵⁾ However, the main constituents in blood and bile sample of rats after oral administration of the extracts of *Polygalae Radix* were analyzed and showed that 3,4,5-trimethoxycinnamic acid (TMCA), methyl 3,4,5-trimethoxycinnamate (M-TMCA) and *p*-methoxycinnamic acid (PMCA) were its bioactive substances.^{6–9)} For example, these compounds were reported to prolong sleeping time induced with hexobarbital in mice⁷⁾; TMCA exhibits anti-stress actions *via* the suppression of norepinephrine content in rat locus coeruleus induced by corticotropin-releasing hormone.¹⁰⁾

In addition, *Polygalae Radix* was also reported to be effective in the treatment for heart palpitations.¹¹⁾ Palpitations are described as unpleasant sensations of irregular and/or forceful beating of the heart, most of which are of cardiac origin although psychiatric disorders, such as anxiety, are also common causes.^{12,13)} Therefore, any type of tachyarrhythmias, regardless of whether or not there is an underlying structural

or arrhythmogenic heart disease, can give rise to palpitations. Particularly, the adrenergic hyperactivation connected with intense emotions and anxiety can, in itself, predispose the patient to supraventricular and/or ventricular arrhythmias.^{14–16)} Thus it is apparently interesting to test whether the bioactive components (such as M-TMCA) exhibit any antiarrhythmic effects. The present study was aimed to investigate the basic electrophysiological effects of these compounds at the cellular level.

MATERIALS AND METHODS

This investigation conforms to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). All animal experimental procedure were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Medicine and Dentistry of New Jersey–New Jersey Medical School and by the Ethical Committee of Xi'an Jiaotong University. All experiments in single myocyte were performed at 35–37°C.

Cell Isolation Single ventricular myocytes were enzymatically isolated from adult rabbit hearts as previously described.^{17,18)} Briefly, New Zealand white rabbits (male, 2 to 3 kg) were anesthetized with intravenous sodium pentobarbital (50 mg/kg) after injecting heparin (200 U/kg, intravenous). The hearts were excised and perfused retrogradely in Langendorff

The authors declare no conflict of interest.

*To whom correspondence should be addressed. e-mail: zzh@mail.xjtu.edu.cn

fashion at 37°C with nominally Ca^{2+} -free Tyrode's solution containing *ca.* 1.4 mg/mL collagenase (type II; Worthington, Biochemical Corp., Lakewood, NJ, U.S.A.) and 0.1 mg/mL protease (type XIV, Sigma, St. Louis, MO, U.S.A.) for 25–30 min. After the enzyme solution was washed out, the hearts were removed from the perfusion apparatus and swirled in a culture dish. The Ca^{2+} concentration was slowly increased to 1.8 mM, and the cells were stored at room temperature and used within 8 h.

Patch-Clamp Methods Myocytes were patch clamped using the whole cell configuration of the patch-clamp technique in the current-clamp or voltage-clamp mode. Voltage or current signals were measured with a MultiClamp 700A patch-clamp amplifier controlled by a personal computer using a Digidata 1322 acquisition board driven by pCLAMP 10 software (Molecular Devices, Sunnyvale, CA, U.S.A.).

To record action potential (AP), patch pipettes (resistance 2 to 4 M Ω) were filled with internal solution containing (in mM): 110 K-aspartate, 30 KCl, 5 NaCl, 10 *N*-(2-hydroxyethyl)-piperazine-*N'*-2-ethanesulfonic acid (HEPES), 0.1 ethylene glycol-bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), 5 MgATP, 5 Na_2 -phosphocreatine, 0.05 cAMP (pH 7.2, adjusted with KOH). Myocytes were superfused with Tyrode's solution containing (in mM): 136 NaCl, 4.0 KCl, 0.33 Na_2PO_4 , 1.8 CaCl_2 , 1 MgCl_2 , 10 glucose and 10 HEPES (pH 7.4, adjusted with NaOH). Action potentials (APs) were elicited with 2-ms, 2- to 4-nA square pulses at a pacing cycle length (PCL) of 2 s.

To record the $I_{\text{Ca,L}}$, patch pipettes were filled with internal solution containing (in mM): 110 Cs-aspartate, 30 CsCl, 5 NaCl, 10 HEPES, 0.1 EGTA, 5 MgATP, 5 Na_2 -phosphocreatine, 0.05 cAMP, (pH 7.2, adjusted with CsOH), and myocytes were perfused with a modified Tyrode's solution in which KCl was replaced with CsCl. The cells were stimulated at a PCL of 2 s with a double-pulse protocol. Following a 100-ms prepulse to -40 mV from the holding potential of -80 mV (to inactivate Na^+ current and T-type Ca^{2+} current), the amplitude of peak $I_{\text{Ca,L}}$ was elicited by a subsequent test depolarization step to 0 mV for 500 ms, and the current-voltage curves were elicited by voltage pulses of 500-ms duration from -40 to 50 mV in 10-mV steps.

To record outward K^+ currents, the pipette and superfusion solutions were the same as those for AP recording. Tetrodotoxin (TTX, 10 μM) and CdCl_2 (0.5 mM) were added into the Tyrode's solution to inhibit I_{Na} and $I_{\text{Ca,L}}$. Total outward K^+ currents were elicited by 3-s voltage pulses from -40 to 50 mV in 10-mV steps at a PCL of 10 s. The amplitudes of peak current ($I_{\text{K,peak}}$) at the beginning, and the steady state current ($I_{\text{K,ss}}$) at the end of the 3-sec depolarizing pulse were evaluated.

Ca^{2+} Transient Measurements Myocytes were loaded with the Ca^{2+} indicator Fluo-4AM (Molecular Probes, Eugene, OR, U.S.A.) by incubating them for *ca.* 30 min in bath solution containing 4 μM Fluo-4 AM and 0.016% pluronic (wt/wt; Molecular Probes, Sigma), after which the cells were washed and placed in a heated chamber on an inverted microscope. Ca^{2+} fluorescence was excited at 485 nm and the emission measured at 515 nm. The fluorescence was monitored using a Nikon Eclipse TE200 inverted microscope with a Fluor x40 oil objective lens (numerical aperture 1.3) and recorded using an Andor Ixon Charge-Coupled Device (CCD) camera (Andor Technology) operating with Imaging Workbench software

(INDEC BioSystems) at *ca.* 100 frames per second with a spatial resolution of 500 $\times$ 400 pixels. Fluorescence intensity was recorded as the ratio F/F_0 of the fluorescence (F) over the basal diastolic fluorescence (F_0).

Chemicals TMCA, PMCA and M-TMCA were obtained from Meryer Chemical Technology Co., Ltd. (Shanghai, China). They were dissolved in dimethylsulfoxide (DMSO) as stock solution (50 mM) before adding to the bath Tyrode's solution at the final concentration. The maximum DMSO concentration was $<0.2\%$ (vol/vol). Other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) unless indicated. All experiments on single myocyte in the present study were carried out at 35–37°C.

Statistical Analysis Data are shown as mean $\pm$ S.D. Statistical differences were evaluated using Student's paired or unpaired *t*-tests or Fisher's exact test, with $p < 0.05$ considered as statistically significant.

RESULTS

The Effect of TMCA, PMCA and M-TMCA on APs in Rabbit Ventricular Myocytes

We first investigated the effects of TMCA, PMCA and M-TMCA on APs in isolated rabbit ventricular myocytes. As shown in Fig. 1, only M-TMCA significantly attenuated action potential duration (APD) in a reversible and concentration-dependent manner (at 15, 30 μM) (Figs. 1C, Dc), while TMCA and PMCA did not show any effect (Figs. 1A, B) at either the same concentrations or a higher concentration (50 μM). The decrease of APD appeared at 30 s–1 min after the application of M-TMCA and reached a steady state at 3–4 min. M-TMCA (30 μM) significantly reduced APD at 50% and 90% repolarization (APD₅₀, APD₉₀) from 219.1 $\pm$ 15.7 and 292.4 $\pm$ 17.3 ms to 184.5 $\pm$ 16.2 and 240.6 $\pm$ 18.5 ms, respectively, (Figs. 1Db, Dc; $n=6$, $p < 0.01$), while the resting membrane potential (RMP), action potential amplitude (APA) and APD at 20% repolarization (APD₂₀) were not changed (Figs. 1C, Da).

The Inhibitory Effect of M-TMCA on L-Type Calcium Current

Since M-TMCA reduced APD₅₀, but not APD₂₀ in rabbit myocytes, we assumed this effect was most likely due to its inhibition on $I_{\text{Ca,L}}$. In the following experiments, we recorded whole-cell $I_{\text{Ca,L}}$ from rabbit ventricular myocytes. As shown in Fig. 2, M-TMCA (30 μM) reduced the amplitude of $I_{\text{Ca,L}}$ at 0.5–1 min after exposure and reached the steady state at 2–3 min (Fig. 2A). Similarly, the inhibitory effect of M-TMCA on $I_{\text{Ca,L}}$ was reversible and concentration-dependent (Figs. 2A, Ba). The mean peak amplitude of $I_{\text{Ca,L}}$ (at 0 mV) was pronouncedly decreased by 15.2 $\pm$ 3.6% from 11.2 $\pm$ 1.1 to 9.2 $\pm$ 0.9 pA/pF at 15 μM ($n=8$, $p < 0.05$) and by 30.3 $\pm$ 6.5% to 7.8 $\pm$ 1.3 pA/pF at 30 μM ($n=5$, $p < 0.01$). The current-voltage relations for the peak current (Fig. 2Bb) showed that the $I_{\text{Ca,L}}$ amplitude was significantly attenuated at testing potentials -10 to $+40$ mV, without any significant voltage shift by M-TMCA.

The Effect of M-TMCA on Ca^{2+} Transients Since the alteration of $I_{\text{Ca,L}}$ would affect Ca^{2+} transient in myocytes, we further investigated the effect of M-TMCA on Ca^{2+} transient. Cells were first paced at a PCL of 2 s under field stimulation. After Ca^{2+} transient reached steady state, cells were perfused with M-TMCA at 5, 15 and 30 μM , respectively, for *ca.* 5 min. M-TMCA concentration-dependently decreased the magnitudes of Ca^{2+} transient (Figs. 3Aa, b). The mean amplitude of

Fig. 1. The Effects of TMCA, PMCA and M-TMCA on Action Potentials (AP) in Rabbit Ventricular Myocytes

Representative AP traces in the absence and presence of 30 μ M TMCA (A), 30 μ M PMCA (B), and 15, 30 μ M M-TMCA and wash out (washing for 2 min) (C) were recorded from three different ventricular myocytes. (D) a–c: Summarized bar graphs showing concentration-dependent shortening effects of M-TMCA on action potential duration at 20%, 50% and 90% repolarization (APD₂₀, APD₅₀, and APD₉₀). **p* < 0.05, ***p* < 0.01 vs. control, *n* = 6.

Fig. 2. Inhibition of $I_{Ca,L}$ by M-TMCA

(A) Time course of peak $I_{Ca,L}$ recorded from a myocyte treated with 30 μ M M-TMCA. Representative traces of $I_{Ca,L}$ corresponding to points a–c are shown under the plot. (Ba) Representative traces of $I_{Ca,L}$ (elicited with a test potential at 0 mV) under control condition, in the presence of 15, 30 μ M M-TMCA and after washout of M-TMCA, respectively. (Bb) The current–voltage relations for $I_{Ca,L}$ under control condition, in the presence of 15 and 30 μ M M-TMCA. **p* < 0.05, ***p* < 0.01 vs. control (*n* = 8).

Ca^{2+} transient was significantly reduced from $F/F_0 = 1.50 \pm 0.27$ (Control) to 1.31 ± 0.15 (15 μ M M-TMCA, *n* = 6, *p* < 0.05) and to 1.24 ± 0.08 (30 μ M M-TMCA, *n* = 6, *p* < 0.01), respectively.

Furthermore, we also observed the effect of M-TMCA on the staircase of Ca^{2+} transient in rabbit myocytes. After Ca^{2+} transient reached steady state, cells were paced with field

Fig. 3. Inhibitory Effects of M-TMCA on Ca^{2+} Transients and Positive Staircase of Ca^{2+} Transients

(Aa) Representative Ca^{2+} transient traces recorded from a myocyte in the absence or presence of 30 μM M-TMCA (3 min after perfusion). (Ab) Ca^{2+} transients were elicited by field stimulation at a pace cycle length (PCL) of 2 s. A summarized bar graph showing the concentration-dependent decreases of Ca^{2+} transients induced by M-TMCA. * $p < 0.05$, ** $p < 0.01$ vs. control, $n = 6$. (Ba) Under control condition, a positive staircase of Ca^{2+} transients was observed. (Bb) Suppression of the positive staircase in the presence of 30 μM M-TMCA. Ca^{2+} transients were elicited by field stimulation at PCLs of 5, 2, 1, 0.8 s, respectively, as indicated. Calcium fluorescence intensity was recorded as the ratio F/F_0 (i.e. the fluorescence (F) over the basal diastolic fluorescence (F_0)) at PCL of 5 s. (Bc) Summarized data ($n = 7$) for Ca^{2+} transient amplitude in the absence or presence of M-TMCA. In the absence of M-TMCA: ## $p < 0.01$ compared to control value at PCL of 5 s. In the presence of M-TMCA: * $p < 0.05$, ** $p < 0.01$ compared to those in the absence of M-TMCA at each PCL.

stimulation at consecutive PCLs of 5 s, 2 s, 1 s, and 0.8 s. As shown in Figs. 3Ba–c, a positive staircase was observed under control condition, whereas in the presence of 30 μM M-TMCA the Ca^{2+} transients were reduced and the positive staircase phenomenon was suppressed.

The Inhibitory Effect of M-TMCA on Afterdepolarizations The early afterdepolarization (EAD), delayed afterdepolarization (DAD) and triggered activity (TA) are major causes for arrhythmias. The increase or reactivation of $I_{\text{Ca,L}}$ has been identified to contribute to the generation of afterdepolarizations, while the inhibition of $I_{\text{Ca,L}}$ suppresses the incidence of EADs and TAs in isolated myocytes. Next, we further investigated the effect of M-TMCA on afterdepolarizations induced under Ca^{2+} overloaded condition (by Isoprenaline and BayK8644) in ventricular myocytes as we previously reported.¹⁹ APs were recorded at a PCL of 2 s. As shown in Fig. 4A, there is no EAD, DAD or TA observed under control condition (Fig. 4Aa). Exposure of isoprenaline (100 nM) plus BayK8644 (50 nM) (Iso/BayK) for 2–3 min consistently caused EADs, DADs or TAs (Fig. 4Ab). Treatment of 30 μM M-TMCA for 3 min in myocytes significantly shortened Iso/

BayK-induced prolongation of APD, completely abolished EADs and TAs (Figs. 4Ac,d). In the presence of M-TMCA (30 μM), the incidence of EAD by counting the number of EADs within 20 consecutive APs was lowered from 95 ± 4.5 to 0% ($n = 5$, $p < 0.01$), the number of DADs or TAs was suppressed from 48 ± 5.9 to $1 \pm 1.2\%$ ($n = 6$, $p < 0.01$). In addition, M-TMCA (30 μM) markedly attenuated the increases of $I_{\text{Ca,L}}$ amplitude (at 0 mV) induced by Iso/BayK from 28.3 ± 5.9 to 18.9 ± 3.8 pA/pF ($n = 5$, $p < 0.01$). The transient inward currents (I_{ti}) induced by Iso/BayK were also eliminated from 0.94 ± 0.08 to 0.02 ± 0.01 pA/pF ($n = 5$, $p < 0.01$) (Figs. 5A, Ba, b).

Little Effect of M-TMCA on Outward K^+ Currents Since the reduction of APD may be also attributed to an increase in outward K^+ currents, we next evaluated the effects of M-TMCA on total outward K^+ currents. As shown in Fig. 6, the current–voltage curves were elicited by voltage pulses of 3-s duration from -40 to 50 mV in 10-mV steps. Neither peak ($I_{\text{K,peak}}$) (Control: 12.5 ± 1.7 vs. M-TMCA: 11.7 ± 1.4 pA/pF, $n = 7$, $p > 0.05$) nor steady state ($I_{\text{K,ss}}$) (Control: 2.4 ± 0.3 vs. M-TMCA: 2.6 ± 0.4 pA/pF, $n = 7$, $p > 0.05$) outward potassium currents were significantly altered by M-TMCA at the concentration of 30 μM that significantly reduced APD under current clamp condition.

DISCUSSION

TMCA, PMCA and M-TMCA have been shown to produce sedative effects and prolong sleeping time in mice,^{7,8}) but their cardiovascular effects remain unclear. In the present study, we have investigated the electrophysiological effects of the bioactive substances of traditional Chinese medicine *Polygalae Radix* on rabbit ventricular myocytes. The major findings are: 1) Among the three bioactive substances of Polygala metabolites, (TMCA, M-TMCA, and PMCA), only M-TMCA exhibits significantly electrophysiological effects on isolated rabbit ventricular myocytes; 2) M-TMCA significantly shortens APD of cardiomyocytes in a concentration-dependent and a reversible manner, which are mainly mediated by the inhibition of $I_{\text{Ca,L}}$; 3) M-TMCA inhibits the intracellular Ca^{2+} transient and suppresses I_{ti} induced by Iso/BayK. 4) M-TMCA suppresses EADs, DADs and TAs induced by Iso/BayK and thus may have antiarrhythmic potentials.

Our results showed that M-TMCA concentration-dependently shortened APD_{50} and APD_{90} . APDD_{20} represents the early phase of action potential repolarization in cardiomyocytes and the fast I_{to} predominately contributes to this repolarizing process. Our results showed that M-TMCA had no significant effect on APD_{20} (Fig. 1), consistent with the result that M-TMCA did not affect fast I_{to} (Fig. 6A). The shortenings of APD_{50} and APD_{90} may implicate a decrease in inward currents (e.g. $I_{\text{Ca,L}}$) or an increase in outward currents (e.g. I_{K}) in the process of cardiomyocyte repolarization. The present study demonstrated that M-TMCA rapidly inhibited $I_{\text{Ca,L}}$ in ventricular myocytes, consistent with the time course for APD_{90} shortening (Fig. 1A). However, M-TMCA at 30 μM , the concentration which dramatically shortened APD and suppressed $I_{\text{Ca,L}}$, did not show any alteration in $I_{\text{K,ss}}$ (Fig. 6). We further showed that M-TMCA inhibited Ca^{2+} transient in a concentration-dependent manner and abolished the positive staircase effect of Ca^{2+} transient in single isolated cardiac myocytes (Fig. 3). It has been suggested that frequency-dependent

Fig. 4. Inhibitory Effects of M-TMCA on Early Afterdepolarizations (EADs), Delayed Afterdepolarizations (DADs), and Triggered Activities (TAs)

(A) Eight consecutive action potentials (APs) recorded from a cell exposed to control perfusate (Aa), 100 nM Isoprenaline plus 50 nM BayK8644 (Iso/BayK) (Ab), Iso/BayK and 30 μ M M-TMCA treatment for 1.5 min (Ac), Iso/BayK and 30 μ M M-TMCA treatment for 3 min (Ad), as well as after washout of M-TMCA (Ae). EADs (*) and DADs (d) and triggered AP (T) are indicated. (B) Bar graphs summarizing the inhibitory effects of M-TMCA on Iso/BayK-induced incidences of EADs (Ba) and DADs/TAs (Bb) within 20 APs ($n=5$). ** $p<0.01$, compared to control, ## $p<0.01$, compared to Iso+BayK group. Fisher's exact test was used.

Fig. 5. Inhibitory Effects of M-TMCA on Iso/BayK-Induced Augment of $I_{Ca,L}$ and I_{ti}

(A) Representative traces of $I_{Ca,L}$ recorded from a cell perfused with control perfusate, 100 nM Iso plus 50 nM BayK8644 (Iso/BayK) and Iso/BayK+30 μ M M-TMCA, respectively. (B) Bar graphs summarizing the inhibitory effects of M-TMCA on Iso/BayK-induced enhancement of $I_{Ca,L}$ (Ba) and I_{ti} (Bb) ($n=5$). ** $p<0.01$, compared to control, ## $p<0.01$, compared to Iso+BayK group.

potentiation of $I_{Ca,L}$ might be an important mechanism underlying positive staircase in cardiomyocytes.^{20,21} It has been evidenced that positive staircase is characterized mainly by an increase in peak current amplitude or slowing inactivation of $I_{Ca,L}$ kinetics.²² Blocking $I_{Ca,L}$ (e.g., verapamil, nifedipine) eliminated the positive staircase of contraction in cardiomyocytes.²³ Our results revealed that M-TMCA significantly suppressed the amplitude of $I_{Ca,L}$ and Ca^{2+} transient, suggesting that the inhibitory effect of M-TMCA on peak $I_{Ca,L}$ plays a critical role in the elimination of the positive staircase of Ca^{2+} transient in rabbit ventricular myocytes.

It was reported that onjisaponin E, F and G in water extract of *Radix Polygalae* inhibited cAMP phosphodiesterase using an *in vitro* screening method,²⁴ which might cause alterations of cAMP metabolism. Although onjisaponin E, F and G, sucrose derivatives and others containing 3,4,5-trimethoxycinnamoyl moiety within their chemical structures in *Polygalae Radix* were metabolized to TMCA and M-TMCA in rat gastrointestinal tract and liver,⁷ it is not known whether M-TMCA itself has the same effect in cardiac myocytes so far. Since our results show that M-TMCA suppresses $I_{Ca,L}$ and decreases Ca^{2+} transients, we assume that M-TMCA should play less effect on phosphodiesterase inhibition in cardiomyocytes. Further studies are needed to clarify this point.

Polygala, often used as a sedative agent, is also applied in the treatment for heart palpitations in traditional Chinese

Fig. 6. Little Effect of M-TMCA on Transient Outward K⁺ Current (I_{to}) and Steady-State Currents ($I_{K,ss}$)

(A) Original current traces in the absence (control) and presence of 30 μ M M-TMCA (M-TMCA). Test potentials ranged from -40 to +50 mV in 10 mV steps (protocol shown in inset). (B) Current-voltage relations of averaged peak I_{to} (Ba) and steady-state currents ($I_{K,ss}$, Bb) showing little effects of M-TMCA on I_{to} and $I_{K,ss}$ ($n=5$).

medical practice. Strictly, a palpitation is an abnormality of heartbeat that ranges from often unnoticed skipped beats or accelerated heart rates to very noticeable changes. Based on our previous studies,¹⁹⁾ therefore, we investigated the effects of M-TMCA on Iso/BayK-induced EADs, DADs, and TAS, which are triggers for arrhythmias at single cell level. Both Iso and BayK potentiate $I_{Ca,L}$. It has been shown that reactivation of $I_{Ca,L}$ is implicated in EAD generation.²⁵⁾ Furthermore, enhanced $I_{Ca,L}$ may lead to Ca²⁺ overload, which consequently results in DADs. In addition, BayK also causes SR Ca²⁺ leaking.²⁶⁾ These effects further induce spontaneous SR Ca²⁺ waves, and subsequently cause DADs and TAS by activating I_{ti} due to electrogenic Na⁺-Ca²⁺ exchange current.^{26,27)} We found that 30 μ M M-TMCA abolished Iso/BayK-induced EADs and significantly reduced the occurrence of DADs and TAS (Fig. 4). Previous studies by our group and others have documented that selective calcium channel blockers (e.g. nifedipine) effectively suppress EADs and DADs in various models.^{19,28)} Consistent with these findings, the suppression of EADs, DADs, and TAS by M-TMCA is most likely mediated by its inhibitory effect on $I_{Ca,L}$. Since the synergistic interactions between $I_{Ca,L}$ and I_{ti} play important roles in EAD generation in the Iso/BayK model,¹⁹⁾ M-TMCA significantly inhibited the augmentation of $I_{Ca,L}$ induced by Iso/BayK, and thereby reduced Ca²⁺ influx and SR Ca²⁺ overload, which account for the suppression of subsequent generation of I_{ti} and EADs, DADs and TAS. It should be noted that we cannot exclude the possibility that M-TMCA may antagonize the β -adrenergic effects *via* the direct blockage of β -adrenergic receptors, which may also suppress the EADs, DADs and TAS induced by Iso/BayK.

To our knowledge, there is no report on the plasma concentrations of M-TMCA in human subjects who take *Polygalae Radix*. However, it has been reported that the plasma concentration of M-TMCA is *ca.* 1–2 μ g/mL (equivalent to *ca.* 4–8 μ M) in rats with single oral administration of water extract of *Polygalae Radix* (5–10 g/kg), and is *ca.* 7–8 μ g/mL (equivalent to *ca.* 28–32 μ M) in rats with intravenous administration of M-TMCA (10 mg/kg).^{7,8)} We assume that the steady-state

plasma concentration of M-TMCA after repetitive administrations would reach the level that is close to its effective concentration range (>10 μ M) for the inhibition of $I_{Ca,L}$ and potential suppression of arrhythmias.

Calcium channel blockers (e.g. verapamil) are used as class IV antiarrhythmic drugs in clinic. Since they preferentially affect the rate of slow response myocardial tissue (*i.e.* the sinoatrial and atrioventricular nodes), calcium channel blockers have been primarily used in the management of certain arrhythmias such as supraventricular tachyarrhythmias associated with atrioventricular nodal reentrant tachycardia,²⁹⁾ as well as in the rate control for atrial fibrillation.³⁰⁾ It has also been demonstrated that calcium channel blockers suppress EADs, DAD, and TAS,^{19,28)} suggesting class IV drugs may be effective in treating afterdepolarization-induced triggered arrhythmias. For example, verapamil is capable of preventing ischemic and reperfusion arrhythmias.³¹⁾ M-TMCA, which exhibits L-type calcium channel inhibitory effect, should also be effective in treating these types of arrhythmias (*i.e.* supraventricular tachyarrhythmias and triggered ventricular arrhythmias) in clinic setting. This notion was supported by the result showing M-TMCA suppressed Iso/BayK-induced EADs, DAD and TAS in isolated myocytes (Fig. 4). Further experiments are needed to fully demonstrate the antiarrhythmic properties of M-TMCA in intact animal models.

Acknowledgements This work was supported by the National Natural Science Foundation of China (81170597) and the Natural Science Foundation of Shaanxi Province (SJ08C223).

REFERENCES

- 1) Yao Y, Jia M, Wu JG, Zhang H, Sun LN, Chen WS, Rahman K. Anxiolytic and sedative-hypnotic activities of polygalasaponins from *Polygala tenuifolia* in mice. *Pharm. Biol.*, **48**, 801–807 (2010).
- 2) Willow M, Carmody J, Carroll P. The effects of swimming in mice on pain perception and sleeping time in response to hypnotic drugs. *Life Sci.*, **26**, 219–224 (1980).

- 3) Sakuma S, Shoji J. Studies on the constituents of the root of *Polygala tenuifolia* WILLDENOW.II. on the structures of onjisaponins A, B and E. *Chem. Pharm. Bull.*, **30**, 810–821 (1981).
- 4) Ikeya Y, Sugama K, Maruno M. Xanthone C-glycoside and acylated sugar from *Polygala tenuifolia*. *Chem. Pharm. Bull.*, **42**, 2305–2308 (1994).
- 5) Ikeya Y, Sugama K, Okada M, Mitsuhashi H. Two xanthones from *Polygala tenuifolia*. *Phytochemistry*, **30**, 2061–2065 (1991).
- 6) Komatsu K, Wakame K, Kano Y. Pharmacological properties of galenical preparation. XVI. Pharmacokinetics of evodiamine and the metabolite in rats. *Biol. Pharm. Bull.*, **16**, 935–938 (1993).
- 7) Wang S, Kozuka K, Saito K, Kano Y. Pharmacological properties of galenical preparations (XVII): Active compounds in blood and bile of rats after oral administrations of extracts of *Polygalae Radix*. *J. Trad. Med.*, **11**, 44–49 (1994).
- 8) Wang S, Kozuka K, Saito K, Komatsu K, Kano Y. Pharmacological properties of galenical preparations (XVIII): Pharmacokinetics of action compounds of *Polygalae Radix*. *J. Trad. Med.*, **11**, 168–175 (1994).
- 9) Wang S, Saito K, Owada E, Kano Y. Pharmacological properties of galenical preparations (XX): Screening of natural prodrugs in *Polygalae Radix*. *J. Trad. Med.*, **12**, 102–108 (1995).
- 10) Kawashima K, Miyako D, Ishino Y, Makino T, Saito K, Kano Y. Anti-stress effects of 3,4,5-trimethoxycinnamic acid, an active constituent of roots of *Polygala tenuifolia* (ONJI). *Biol. Pharm. Bull.*, **27**, 1317–1319 (2004).
- 11) Chang HM, But PP-H. “Pharmacology and applications of Chinese material medica,” Vol. 1, ed. by World Scientific Publishing, Singapore, pp. 551–553 (1986).
- 12) Wexler RK, Pleister A, Raman S. Outpatient approach to palpitations. *Am. Fam. Physician*, **84**, 63–69 (2011).
- 13) Reagan BW, Huang RL, Clair WK. Palpitations: an annoyance that may require clairvoyance. *Circulation*, **125**, 958–965 (2012).
- 14) Nyrnes A, Mathiesen EB, Njølstad I, Wilsgaard T, Løchen ML. Palpitations are predictive of future atrial fibrillation. An 11-year follow-up of 22,815 men and women: the Tromsø Study. *Eur. J. Prev. Cardiol.* (2012) May 15. [Epub ahead of print].
- 15) Lampert R, Joska T, Burg MM, Batsford WP, McPherson CA, Jain D. Emotional and physical precipitants of ventricular arrhythmia. *Circulation*, **106**, 1800–1805 (2002).
- 16) Ziegelstein RC. Acute emotional stress and cardiac arrhythmias. *JAMA*, **298**, 324–329 (2007).
- 17) Xie LH, Weiss JN. Arrhythmogenic consequences of intracellular calcium waves. *Am. J. Physiol. Heart Circ. Physiol.*, **297**, H997–H1002 (2009).
- 18) Zhao Z, Fefelova N, Shanmugam M, Bishara P, Babu GJ, Xie LH. Angiotensin II induces afterdepolarizations via reactive oxygen species and calmodulin kinase II signaling. *J. Mol. Cell. Cardiol.*, **50**, 128–136 (2011).
- 19) Zhao Z, Wen H, Fefelova N, Allen C, Baba A, Matsuda T, Xie LH. Revisiting the ionic mechanisms of early afterdepolarizations in cardiomyocytes: predominant by Ca waves or Ca currents? *Am. J. Physiol. Heart Circ. Physiol.*, **302**, H1636–H1644 (2012).
- 20) Palomeque J, Vila Petroff MG, Mattiazzi A. Pacing staircase phenomenon in the heart: from Bodwitch to the XXI century. *Heart Lung Circ.*, **13**, 410–420 (2004).
- 21) Endoh M. Force-frequency relationship in intact mammalian ventricular myocardium: physiological and pathophysiological relevance. *Eur. J. Pharmacol.*, **500**, 73–86 (2004).
- 22) Richard S, Perrier E, Fauconnier J, Perrier R, Pereira L, Gómez AM, Bénitah JP. ‘Ca²⁺-induced Ca²⁺ entry’ or how the L-type Ca²⁺ channel remodels its own signalling pathway in cardiac cells. *Prog. Biophys. Mol. Biol.*, **90**, 118–135 (2006).
- 23) Brotto MA, Creazzo TL. Ca²⁺ transients in embryonic chick heart: contributions from Ca²⁺ channels and the sarcoplasmic reticulum. *Am. J. Physiol.*, **270**, H518–H525 (1996).
- 24) Nikaido T, Ohmoto T, Saitoh H, Sankawa U, Sakuma S, Shoji J. Inhibitors of cyclic adenosine monophosphate phosphodiesterase in *Polygala tenuifolia*. *Chem. Pharm. Bull.*, **30**, 2020–2024 (1982).
- 25) January CT, Riddle JM. Early afterdepolarizations: mechanism of induction and block. A role for L-type Ca²⁺ current. *Circ. Res.*, **64**, 977–990 (1989).
- 26) Mackiewicz U, Emanuel K, Lewartowski B. Agonist of dihydropyridine receptors, BayK8644 depresses excitation-contraction coupling in myocytes of guinea pig heart. *J. Physiol. Pharmacol.*, **52**, 459–469 (2001).
- 27) Sung RJ, Lo CP, Hsiao PY, Tien HC. Targeting intracellular calcium cycling in catecholaminergic polymorphic ventricular tachycardia: a theoretical investigation. *Am. J. Physiol. Heart Circ. Physiol.*, **301**, H1625–H1638 (2011).
- 28) Yamada M, Ohta K, Niwa A, Tsujino N, Nakada T, Hirose M. Contribution of L-type Ca²⁺ channels to early afterdepolarizations induced by IKr and IKs channel suppression in guinea pig ventricular myocytes. *J. Membr. Biol.*, **222**, 151–166 (2008).
- 29) Haines DE, DiMarco JP. Current therapy for supraventricular tachycardia. *Curr. Probl. Cardiol.*, **17**, 411–477 (1992).
- 30) Dorian P. Rate control in atrial fibrillation. *N. Engl. J. Med.*, **362**, 1439–1441 (2010).
- 31) Singh BN, Nademanee K. Use of calcium antagonists for cardiac arrhythmias. *Am. J. Cardiol.*, **59**, 153B–162B (1987).