Taurine-magnesium Coordination Compound Attenuates Hypoxia/Reoxygenation Induced Ion Channel Dysfunction in Rat Ventricular Myocytes

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Key words
- arrhythmia
- ion channels
- hypoxia/reoxygenation
- rat
- myocytes

Abstract

Because of the known anti-arrhythmic effects of taurine-magnesium coordination compound (TMCC), the aim of the present study was to explore the electrophysiological effects of TMCC on hypoxia/reoxygenation (H/R)-induced arrhythmias in rat ventricular myocytes. Sodium current (I_{Na}), the L-type calcium current ($I_{Ca,L}$), and the transient outward potassium current (I_{to}) were evaluated using whole-cell patch-clamp recordings in rat ventricular myocytes following H/R injury. The H/R group significantly decreased sodium currents, while L-type calcium currents and transient outward potassium currents was significantly increased (all $p<0.01$). TMCC (200 and 400 µM) prevented abnormal sodium currents induced by H/R by inhibiting steady-state inactivation. It also counteracted abnormal L-type calcium currents induced by H/R by inhibiting steady-state activation and facilitating steady-state inactivation. In addition, it mitigated abnormal transient outward potassium currents induced by H/R by inhibiting steady-state activation. TMCC prevents H/R-induced arrhythmias in rat ventricular myocytes by modifying ion channel function.

Introduction

Taurine and magnesium are effective supplements to prevent arrhythmias and protect the myocardium [1–3]. Taurine blocks voltage-dependent L-type calcium channels, which reduces calcium channel current density and decreases pathological and drug-induced arrhythmia incidences [4,5]. Taurine produces a positive inotropic effect by increasing intracellular Ca^{2+} by activating the taurine-Na^+ co-transporter and Na^+-Ca^{2+} exchanger [6,7]. Magnesium, a natural calcium antagonist, inhibits Ca^{2+} influx through both L-type and N-type calcium channels [8]. Extracellular magnesium significantly inhibits Ca^{2+} influx, when extracellular calcium levels fall below physiological concentrations [9].

Our laboratory has previously shown that the combined use of taurine and magnesium was superior to either alone to treat arrhythmias induced by ischemia/reperfusion. Based on these findings, we chemically synthesized a taurine-magnesium coordination compound (TMCC, **Fig. 1**). Over the years, TMCC has become recognized as a specific and selective antiarrhythmic agent with a low toxicity profile. Arrhythmias induced by electrical stimulation, epinephrine, aconitine, stophanthin G, or cesium choride have all been blocked by TMCC pre-treatment [10]. TMCC markedly inhibited I_{Na} and I_{to}, and moderately stimulated cardiac $I_{Ca,L}$ in rat ventricular myocytes. These results implicate I_{Na}, $I_{Ca,L}$, and I_{to} as targets of the antiarrhythmic effects of TMCC [11].

Ischemia/reperfusion (I/R) in vivo or hypoxia/reoxygenation (H/R) in vitro induces endothelial dysfunction, reactive oxygen species, abnormal lipid metabolism, calcium overload, and apoptosis [12,13]. Our laboratory has previously shown that TMCC shortens the duration of arrhythmia induced by ischemia. However, little is known about the effects of TMCC on abnormal ionic channels induced by H/R. Based on the previous results, we used H/R injury to stimulate transmembrane ion channels and disrupt channel equilibrium. The efficacy of TMCC on abnormal ion channels to amiodarone, a commonly used antiarrhythmic agent, was examined.

Methods

Animals

All experiments were carried out according to the guidelines of the local ethics committee at our institution, and our protocol was approved

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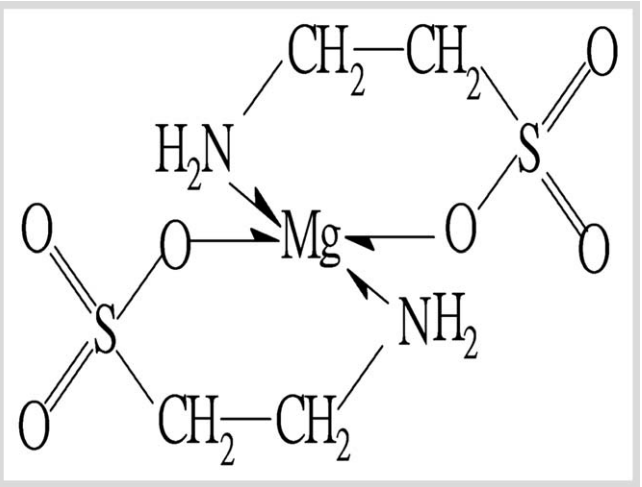

Fig. 1 Structure of taurine-magnesium coordination compound.

by the committee. Wistar rats of either sex, weighing 200–250 g (grade II, certificate no. 2008–0002) were purchased from the Experimental Animal Center of China Tianjin Medical University. Data was acquired from at least n = 6 myocytes from 1 rat for each experiment.

Drugs

TMCC was kindly provided by the Department of Chemistry, Tianjin Medical University (batch no. 100031) and was dissolved in distilled water to prepare a stock solution (200 mM). The stock solution was diluted with Tyrode's solution for the patch clamp studies.

Solutions

The Tyrode solution contained 126 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.33 mM NaH_2PO_4, 10 mM glucose, and 10 mM HEPES, and the pH was adjusted to 7.4 with NaOH. The nominally Ca^{2+}-free Tyrode solution was prepared by removing $CaCl_2$ from the Tyrode solution. Krebs solution was used to store cells. Krebs solution consisted of 70 mM glutamic acid, 15 mM taurine, 30 mM KCl, 10 mM KH_2PO_4, 0.5 mM $MgCl_2$, 0.5 mM Ethylene glycol bis(2-aminoethyl) tetraacetic acid (EGTA), 10 mM HEPES, 10 mM glucose, and 1 % albumin, and the pH was adjusted to 7.4 with KOH. The pipette solution contained 20 mM KCl, 110 mM potassium aspartate, 5 mM HEPES, 1 mM $MgCl_2$, 10 mM EGTA, 5 mM Na_2 -ATP, and the pH was adjusted to 7.2 with KOH. The pipette solution for recording $I_{ca,L}$ contained 20 mM CsCl, 110 mM cesium aspartate, 1 mM $MgCl_2$, 5 mM HEPES, 10 mM EGTA, and 5 mM Na_2-ATP, and the pH was adjusted to 7.2 with CsOH. The standard bath solution for recording $I_{ca,L}$ contained 126 mM choline chloride, 5.4 mM CsCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.33 mM NaH_2PO_4, 10 mM glucose, and 10 mM HEPES, and the pH was adjusted to 7.4 with CsOH. Type II collagenase, EGTA, taurine, and bovine serum albumin (BSA) were all purchased from Sigma Chemical Co (St. Louis, MO, USA).

Cell preparation

As described previously, we isolated single ventricular myocytes from adult rat left ventricles by enzymatic dissociation [11]. Briefly, rats were anesthetized by intraperitoneal injection of sodium pentobarbital ($50\,mg\cdot kg^{-1}$) and heparin ($300\,U\cdot kg^{-1}$). The rat heart was excised, placed on a Langendorff apparatus, and perfused in a retrograde fashion with oxygenated ice-cold Ca^{2+}-free Tyrode's solution through the aorta at a perfusion rate of $4\,ml\cdot min^{-1}$ for 5 min. The heart was subsequently perfused with Ca^{2+}-free Tyrode's solution with 34 µM $CaCl_2$ and $300\,mg\cdot L^{-1}$ collagenase II at 37 °C for 12 min. The left ventricle was removed, cut into 1 mm pieces, and placed in Krebs solution. Single myocytes were harvested by passing the single cell suspension through a nylon mesh with pore size of 200 µm. The cells were rested by incubating in Krebs solution at room temperature for 1–3 h. The Ca^{2+} concentration in the Krebs solution was gradually increased to 1 mM before the experiment.

Whole cell patch clamp experiments

For whole cell patch clamp experiments, the isolated ventricular myocyte suspension was pipetted into a perfusion chamber that was set on the stage of an inverted microscope (Olympus IX51, Tokyo, Japan). Glass microelectrodes were made as described previously [11]. The ion currents, including sodium, L-type calcium, and transient outward potassium currents, were recorded with an Axopatch 700B amplifier (Axon Instruments, Foster City, CA, USA). The recordings were digitized at 2–10 kHz with the Digit Data 1440A analogue-to-digital converter (Axon Instruments), and the pCLAMP 10.0 software (Axon Instruments) was used to acquire data and analyze traces for the voltage clamp protocols. The whole-cell series resistance was compensated to > 80 %, and the current traces were presented as current density ($pApF^{-1}$). All experiments were performed at room temperature (23 ± 1 °C).

Using the above protocols, whole cell I_{Na} was tested at potentials ranging from −120 to +30 mV, at 10 mV increments. The I_{Na} was calculated as the difference between peak inward current and the holding current level. Steady-state activation curves of I_{Na} were derived from the current-voltage relationship and were fitted according to the Boltzmann equation. The voltage dependent steady-state inactivation of I_{Na} was defined by applying a 50 ms conditioning pulse, ranging from −150 mV to +30 mV in 10 mV increments, followed by a 25 ms test pulse to −30 mV. The data were fitted using the Boltzmann distribution equation. Whole cell $I_{Ca,L}$ was elicited from a holding potential of −40 mV and tested with potentials ranging from −40 to+60 mV, in 10 mV increments. $I_{Ca,L}$ was calculated as the difference between peak inward and holding current. The steady-state activation curve of $I_{Ca,L}$ was derived from the current-voltage relationships and fitted to the Boltzmann equation. The voltage dependent steady-state inactivation of $I_{Ca,L}$ was determined by applying a 1 000 ms conditioning pulse ranging from –40 to+20 mV, in 10 mV increments, followed by a 150 ms test pulse to 0 mV, with a holding potential of −40 mV. The data were fitted to the Boltzmann distribution equation. Whole cell I_{to} was elicited from a holding potential of −50 mV, with test potentials ranging from−50 to+65 mV, in 5 mV increments. The outward peak amplitude was I_{to}, which was the major repolarizing potassium current in rat ventricular myocytes. The steady-state activation curve of I_{to} was derived from the current-voltage relationship and fitted according to the Boltzmann equation. The voltage dependent steady-state inactivation of I_{to} was calculated by applying 500 ms prepulses from −120 to +30 mV prior to pulsing to a Vm of+60 mV. Steady-state inactivation curves were constructed by normalizing the measured I_{to} after each prepulse (I/Imax) and plotting the values against the prepulse voltage. The curves were fitted to the Boltzmann equation to derive steady-state inactivation parameters $V_{1/2}$ and k.

Table 1 Effects of taurine-magnesium coordination compound (TMCC) and amiodarone on the currents, steady-state activation and inactivation of abnormal sodium channel induced by hypoxia/reoxygenation (H/R) in rat isolated ventricular myocytes.

Groups	I_{Na} (pA/pF)	$V_{1/2(act)}$/mV	K_{act}	$V_{1/2(inact)}$/mV	K_{inact}
control	−56.89±2.07##	−82.98±1.64#	4.13±0.61	−127.04±1.83##	5.90±0.55#
H/R	−35.05±1.52**	−74.94±3.74*	3.75±0.59	−140.71±1.25**	8.16±0.98*
H/R+200 μM TMCC	−41.52±0.86**##	−77.21±2.57*	4.45±0.54	−137.41±1.14**#	7.78±0.84*
H/R+400 μM TMCC	−48.34±0.99**##	−78.82±1.44*	4.75±0.63	−133.06±2.99#	7.42±0.87
H/R+40 μM amiodarone	−39.44±1.24**##	−78.88±1.19*	4.86±0.31	−136.25±1.54**##	8.35±0.78**

n=6 per group; *P<0.05 vs. control group, **P<0.01 vs. control group, # P<0.05 vs. H/R group, ## P<0.01 vs. H/R group

To record the sodium current, cadmium chloride (0.3 mM) was added to the external solution to block the $I_{Ca\text{-}L}$ current, and the maximum sodium current ($I_{Na,max}$) was observed at −60 mV. To record the calcium current, the magnitude of $I_{ca,L}$ was measured as the peak inward current and the maximum calcium current ($I_{ca,L,max}$) was observed at 15 mV. To record transient outward potassium currents, 0.2 mM Cd^{2+} and 0.2 μM Ba^{2+} were used to block $I_{ca,L}$ and I_{k1}, respectively, the maximum transient outward potassium current ($I_{to,max}$) was observed at 50 mV. The standard bath solution was replaced with a bath solution filled with N_2 to simulate hypoxia. After 15 min of hypoxia, the N_2 bath solution was replaced with a bath solution filled with O_2 to simulate reperfusion. The change in densities of $I_{Na,max}$, $I_{ca,L,max}$ and $I_{to,max}$ were measured after single ventricular myocytes were exposed to H/R for 10 min. TMCC was administered after exposed to H/R and the $I_{Na,max}$, $I_{ca,L,max}$ and $I_{to,max}$ were measured 10 min after treatment.

Statistical analysis

Data were presented as mean±standard error of the mean. Curve fitting was made using pCLAMP 10.0 (Axon Instruments) or software Origin 6.0 (Microcal Software, Northampton, MA, USA). Statistical significance was analyzed using a 2-tailed paired Student's *t*-test for comparisons of 2 means or analysis of variance (ANOVA) for comparison of multiple means. A *P* value <0.05 was considered statistically significant.

Results

TMCC prevented abnormal sodium channels induced by H/R in rat ventricular myocytes

As shown in **Table 1**, at a −60 mV test pulse, sodium current density decreased from −56.89±2.07 $pApF^{-1}$ to −35.05±1.52 $pApF^{-1}$ (n=6 per group, P<0.01 vs. control). Following H/R injury, the peak inward sodium current decreased by 38% (P<0.05 vs. control). TMCC (200 or 400 μM) or amiodarone (40 μM) restored the decreased sodium currents induced by H/R from −35.05±1.52 $pApF^{-1}$ to −41.52±0.86 $pApF^{-1}$, −48.34±0.99 $pApF^{-1}$ and −39.44±1.24 $pApF^{-1}$, respectively. (n=6 per group, *P*<0.01 vs. H/R).

As shown in **Table 1**, **Fig. 2g, h**, steady-state activation and inactivation curves of the abnormal sodium channels obtained following H/R injury before and after TMCC treatment. The H/R group shifted the half activation potential from −82.98±1.64 mV to −74.94±3.74 mV (n=6 per group, *P*<0.05 vs. control), and the slope parameter (κ) was not affected. Neither TMCC nor amiodarone shifted any of the activation parameters. The H/R group shifted the half inactivation potential from −127.04±1.83 mV to −140.71±1.25 mV (n=6 each group, *P*<0.01 vs. control) and the slope parameter (κ) from 5.90±0.55 mV to 8.16±0.98 mV (n=6 per group, *P*<0.05 vs. control). TMCC or amiodarone shifted the half inactivation potential from −140.71±1.25 mV to −137.41±1.14 mV, −133.06±2.99 mV (n=6 per group, *P*<0.05 vs. H/R) and −136.25±1.54 mV (n=6 per group, *P*<0.01 vs. H/R). However, the slope parameter (κ) was not changed significantly between the H/R group and any of the drug treatment groups.

TMCC prevented abnormal L-type calcium channels induced by H/R in rat ventricular myocytes

As shown in **Table 2**, at a 10 mV test pulse, the calcium current density increased from −3.35±0.62 $pApF^{-1}$ to −5.69±0.25 $pApF^{-1}$ (n=6 per group, *P*<0.01 vs. control). Following H/R injury, the peak inward calcium currents increased significantly by 41% (p<0.05 vs. control). TMCC or amiodarone restored the abnormal calcium currents by increasing the currents from −5.69±0.25 $pApF^{-1}$ to −4.41±0.22 $pApF^{-1}$ and −3.82±0.21 $pApF^{-1}$ for TMCC (at the 200 and 400 μM concentrations, respectively) and to −3.66±0.27 $pApF^{-1}$ for amiodarone (n=6 per group, *P*<0.01 vs. H/R).

The steady-state activation and inactivation curves of abnormal calcium channels obtained following H/R injury before and after TMCC treatment are shown in **Table 2**, **Fig. 3g, h**. The H/R group shifted the half activation potential from −12.63±0.69 mV to −17.12±0.65 mV (n=6 per group, *P*<0.01 vs. control) and the slope parameter (κ) from 7.10±0.48 mV to 8.84±0.47 mV (n=6 per group, *P*<0.01 vs. control). TMCC or amiodarone shifted the half activation potential from −17.12±0.65 mV to −14.63±0.85 mV and −12.87±1.09 mV for TMCC (at the 200 and 400 μM concentrations, respectively) and to −13.15±0.84 mV for amiodarone (n=6 per group, *P*<0.01 vs. H/R). However, the slope parameter (κ) was not significantly changed. The H/R group shifted the half inactivation potential from −22.67±0.73 mV to −16.24±0.89 mV (n=6 per group, *P*<0.01 vs control), and the slope parameter (κ) from 7.60±0.35 mV to 5.80±0.75 mV (n=6 per group, *P*<0.05 vs. control). TMCC or amiodarone shifted the half inactivation potential from −16.24±0.89 mV to −20.76±0.29 mV and −22.77±0.75 mV for TMCC (at the 200 and 400 μM concentrations, respectively) and to −22.10±0.40 mV for amiodarone (n=6 per group, all *P*<0.01 vs. H/R). However, the slope parameter (κ) was not significantly changed.

TMCC prevented abnormal transient outward potassium channels induced by H/R in rat ventricular myocytes

As shown in **Table 3**, at a 50 mV test pulse, the $I_{to,max}$ density was increased from 8.40±0.66 $pApF^{-1}$ to 13.50±0.41 $pApF^{-1}$ (n=6 per group, *P*<0.01 vs. control). Following H/R injury, the peak current density of the transient outward potassium currents was significantly increased by 38%. TMCC (at the 200 and 400 μM concentrations) or amiodarone prevented the increase

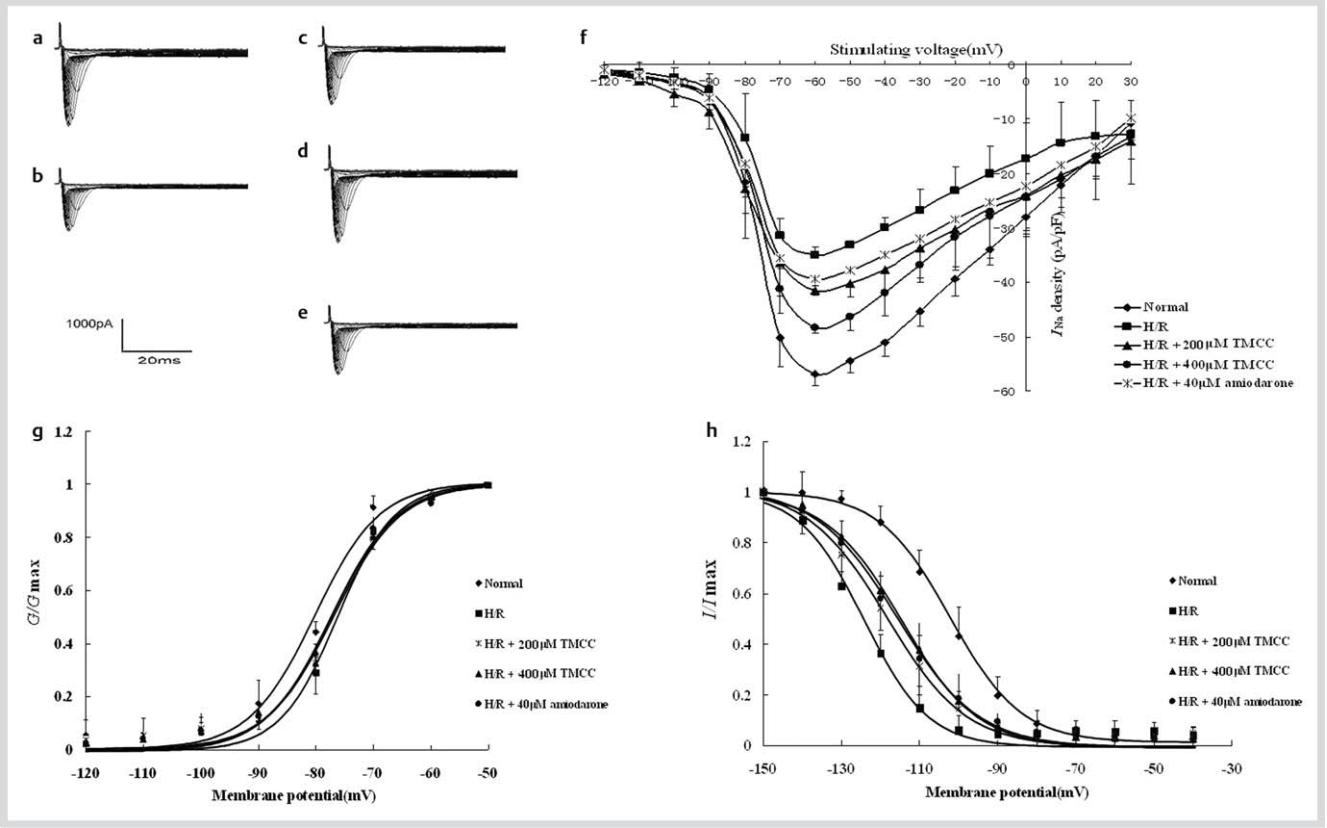

Fig. 2 TMCC effects on abnormal sodium channels induced by H/R in rat ventricular myocytes. **a**, **b**, **c**, **d** and **e** are representative traces of I_{Na} recorded in control conditions **a**, H/R **b**, after the addition of H/R + 200 μM TMCC **c**, H/R + 400 μM TMCC **d**, and H/R + 40 μM amiodarone **e**. **f** I – V relations for I_{Na} of control, H/R, H/R + TMCC (200 μM), H/R + TMCC (400 μM) and H/R + amiodarone (40 μM). **g** and **h** effects of TMCC (200 μM and 400 μM) and amiodarone (40 μM) on steady-state activation **g** and inactivation **h** of abnormal sodium currents induced by H/R in rat isolated ventricular myocytes. Sample sizes are n = 6 per group; $P < 0.01$ vs. control.

Table 2 Effects of taurine-magnesium coordination compound (TMCC) and amiodarone on the currents, steady-state activation and inactivation of abnormal L-type calcium channel induced by hypoxia/reoxygenation (H/R) in rat isolated ventricular myocytes.

Groups	$I_{Ca,L}$ (pA/pF)	$V_{1/2(act)}$/mV	K_{act}	$V_{1/2(inact)}$/mV	K_{inact}
control	−3.35± 0.50##	−12.63±0.69##	7.10±0.48##	−22.67±0.73##	7.60±0.35#
H/R	−5.69±0.25**	−17.12±0.65**	8.84±0.47**	−16.24±0.89**	5.80±0.75*
H/R + 200 μM TMCC	−4.41±0.22*##	−14.63±0.85*##	8.11±0.59	−20.76±0.29*##	6.19±0.98
H/R + 400 μM TMCC	−3.82±0.21##	−12.87±1.09##	7.57±0.18	−22.77±0.75##	6.23±0.98
H/R + 40 μM amiodarone	−3.66±0.27##	−13.15±0.84##	7.50±0.70	−22.10±0.40##	6.54±0.62

n=6 per group; *P<0.05 vs. control group, **P<0.01 vs. control group, #P<0.05 vs. H/R group, ##P<0.01 vs. H/R group

in transient outward potassium currents induced by H/R from 13.50±0.41 $pApF^{-1}$ to 10.60±0.84 $pApF^{-1}$ and 7.80±0.15 $pApF^{-1}$ for the 200 and 400 μM concentrations of TMCC, respectively, and to 7.93±0.43 $pApF^{-1}$ for amiodarone (n = 6 per group, all $P < 0.01$ vs. H/R).

The steady-state activation and inactivation curves of abnormal transient outward potassium channels obtained following H/R injury before and after TMCC treatment are shown in **Table 3**, **Fig. 4g, h**. The H/R group shifted the half activation potential from 25.51±1.58 mV to 21.80±1.03 mV (n = 6, $P < 0.01$ vs. control), but the slope parameter (κ) was not significantly changed. TMCC or amiodarone shifted the half activation potential from 21.80±1.03 mV to 23.33±2.92 mV and 27.04±0.60 mV for TMCC and to 24.17±1.06 mV for amiodarone (n = 6, $P < 0.01$ vs. H/R), but the slope parameter (κ) was not significantly changed. The half inactivation potential and the slope parameter (κ) were also not significantly changed between the H/R group and any of the drug treatment groups.

Discussion

TMCC has been used effectively as an antiarrhythmic agent in vivo. The mechanisms underlying its actions, however, have not been fully described. The major findings of the current study were TMCC prevented abnormal sodium channel, L-type calcium channel and transient outward potassium channel induced by H/R in single rat ventricular myocytes.

Ischemia/reperfusion injury is known to stimulate structural and functional remodeling of the left ventricle. This remodeling enhances the arrhythmic potential of the myocardium and increases the chance of sudden cardiac death. Both the ischemic and reoxygenation phases perturb normal cardiac myocyte function. Previous studies have assigned roles for K^+ channels, voltage-dependent L-type Ca^{2+} channels, Na^+/Ca^{2+} exchanger, and sarcoplasmic reticulum Ca^{2+}-ATPase pump, and ryanodine receptors [14–19]. Our previous studies showed that TMCC could inhibit sodium currents and transient outward potassium

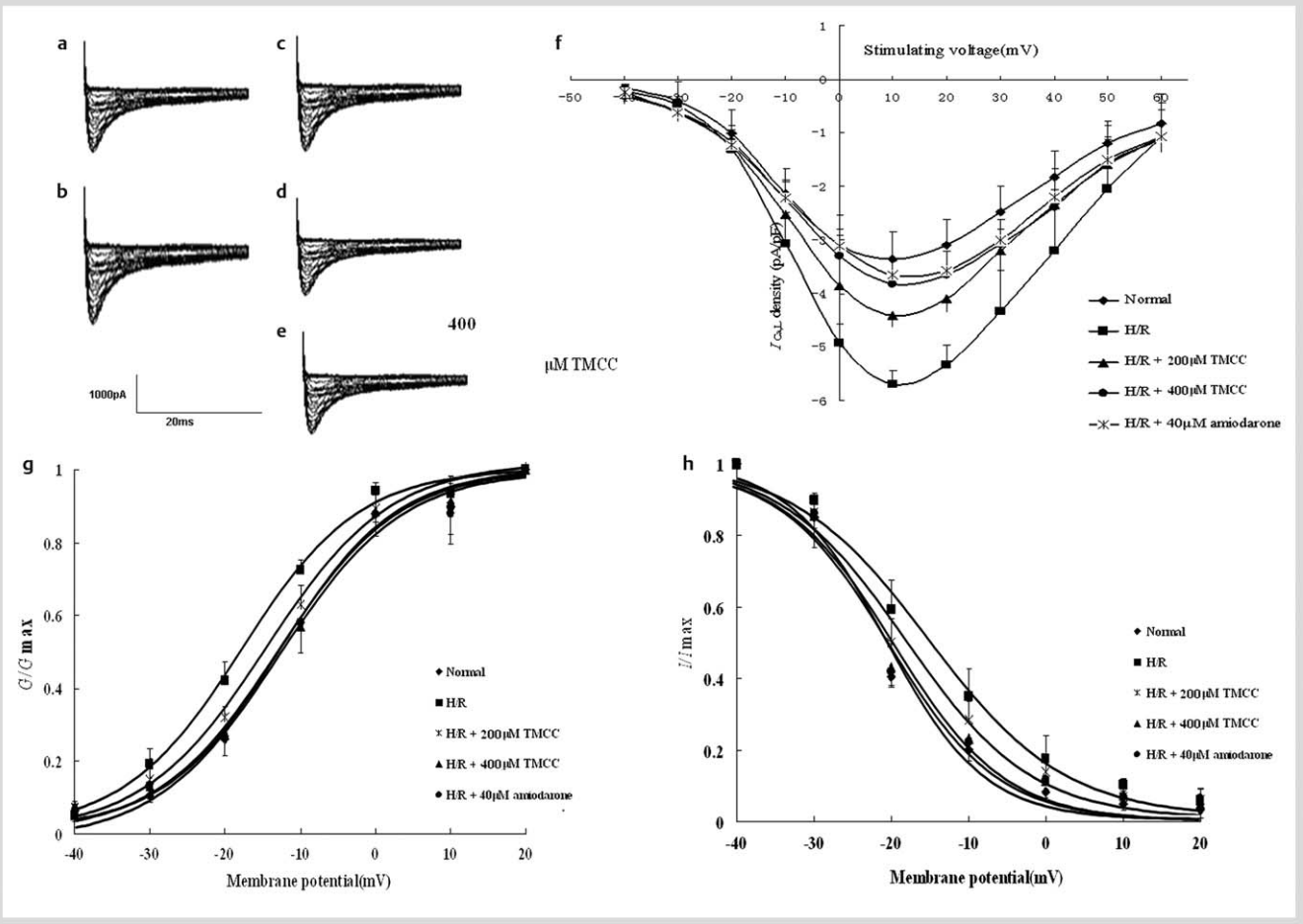

Fig. 3 TMCC effects on abnormal L-type calcium channels induced by H/R in rat ventricular myocytes. **a**, **b**, **c**, **d** and **e** are representative traces of $I_{Ca,L}$ recorded in control conditions **a**, H/R **b**, after the addition of H/R + 200 μM TMCC **c**, H/R + 400 μM TMCC **d**, and H/R + 40 μM amiodarone **e**. **f** I – V relations for $I_{Ca,L}$ of control, H/R, H/R + TMCC (200 μM), H/R + TMCC (400 μM) and H/R + amiodarone (40 μM). **g** and **h** effects of TMCC(200 μM and 400 μM) and amiodarone (40 μM) on steady -state activation **g** and inactivation **h** of abnormal L-type calcium currents induced by H/R in rat isolated ventricular myocytes. Sample sizes are n = 6 per group and $P < 0.01$ vs. control.

Table 3 Effects of taurine-magnesium coordination compound (TMCC) and amiodarone on the currents, steady-state activation and inactivation of abnormal transient outward potassium channel induced by hypoxia/reoxygenation (H/R) in rat isolated ventricular myocytes.

Groups	I_{to} (pA/pF)	$V_{1/2(act)}$/mV	K_{act}	$V_{1/2(inact)}$/mV	K_{inact}
control	8.40 ± 0.66##	25.51 ± 1.58##	10.89 ± 0.77	−29.50 ± 0.78	3.98 ± 0.35
H/R	13.50 ± 0.41**	21.80 ± 1.03**	11.02 ± 0.43	−29.33 ± 0.56	3.89 ± 0.31
H/R + 200 μM TMCC	10.60 ± 0.84**##	23.33 ± 2.92##	11.43 ± 0.67	−30.96 ± 0.19	3.92 ± 0.29
H/R + 400 μM TMCC	7.80 ± 0.15##	27.04 ± 0.60##	11.44 ± 0.33	−30.45 ± 1.07	3.93 ± 0.27
H/R + 40 μM amiodarone	7.93 ± 0.43##	24.17 ± 1.06##	10.79 ± 0.70	−30.98 ± 0.79	3.95 ± 0.62

n=6 per group; **P<0.01 vs. control group, ##P<0.01 vs. H/R group

currents, concomitant with a moderate increase in L-type calcium currents [11]. From that study, we concluded that I_{Na}, $I_{ca,L}$, and I_{to} may be key factors involved in the development arrhythmias. Here, we extend the previous study to determine the effects of TMCC on these abnormal channels induced hypoxia/reoxygenation.

The excitability of cardiac ventricular myocytes is critically regulated by the voltage-gated Na^+ channel. As such, the voltage-gated Na^+ channel is a primary target of several neurotoxins as well as therapeutic agents. Therapeutic agents that have been shown to be efficacious against the voltage-gated Na^+ channel include quinidine, lidocaine, and phenytoin [20]. Our previous study showed that I_{Na} was blocked by TMCC in a concentration-dependent manner, and the effects of TMCC (400 μM) were equal to that of amiodarone. TMCC inhibited I_{Na} through retardation of steady-state activation and steady-state inactivation [11]. Our results showed that sodium currents were significantly decreased by H/R, which shifted steady-state activation curves to the right and inactivation curves to the left. These results suggest that the steady-state activation of the Na^+ channel was decelerated while the inactivation was accelerated. The H/R group may inhibit I_{Na} by blocking the steady-state activation and facilitating the steady-state inactivation. TMCC or amiodarone restored sodium currents significantly. The effect of amiodarone, however, was not as potent as the effect of TMCC. After H/R injury, TMCC or amiodarone shifted the steady-state inactivation curves to the right, while TMCC or amiodarone failed to alter the steady-state activation curves, suggesting that the

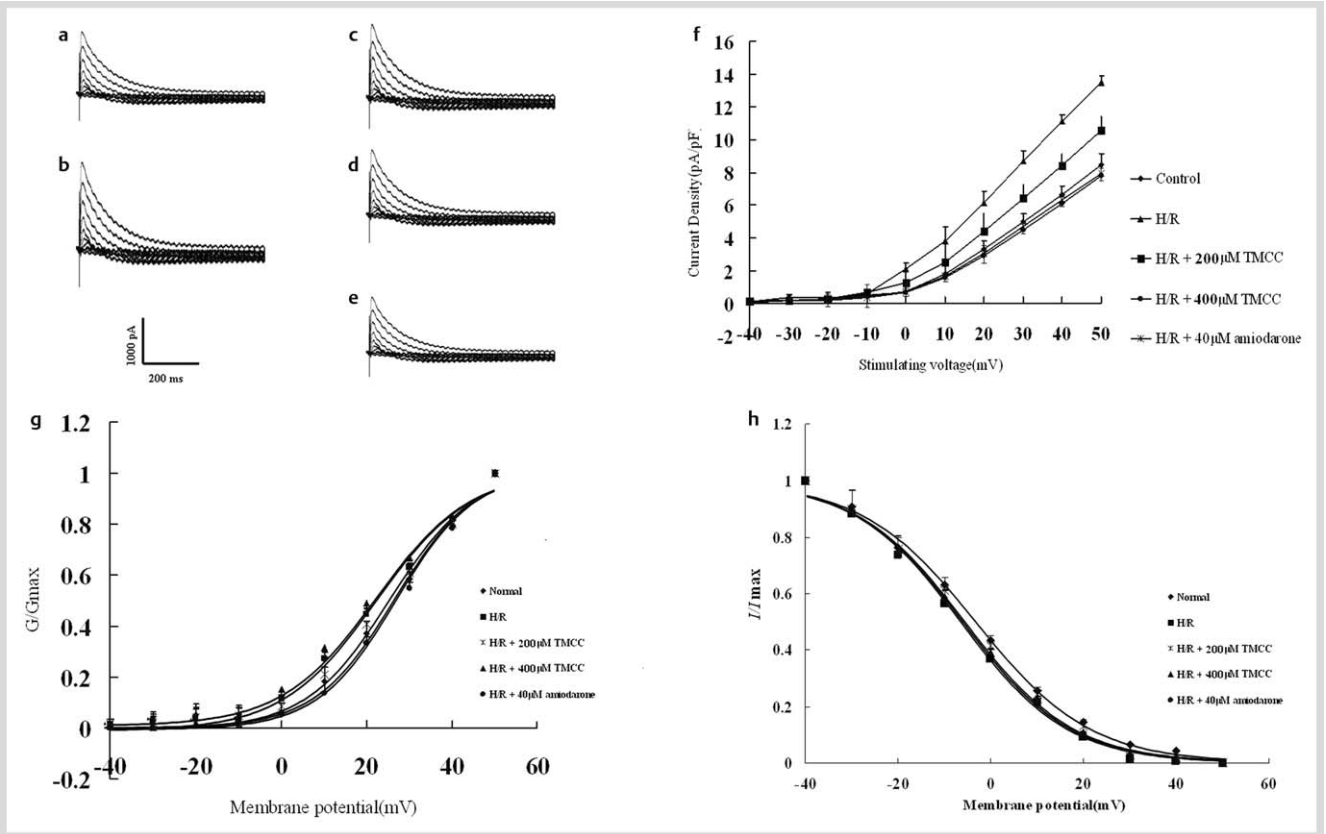

Fig. 4 TMCC effects on abnormal transient outward potassium channels induced by H/R in rat ventricular myocytes. **a**, **b**, **c**, **d** and **e** are representative traces of I_{to} recorded in control conditions **a**, H/R **b**, after the addition of H/R + 200 μM TMCC **c**, H/R + 400 μM TMCC **d**, and H/R + 40 μM amiodarone **e**. **f** I – V relations for I_{to} of control, H/R, H/R + TMCC (200 μM), H/R + TMCC (400 μM) and H/R + amiodarone (40 μM). **g** and **h** effects of TMCC (200 μM and 400 μM) and amiodarone (40 μM) on steady-state activation **g** and inactivation **h** of abnormal transient outward potassium currents induced by H/R in rat isolated ventricular myocytes. Sample sizes are n = 6 per group and $P < 0.01$ vs. control.

steady-state inactivation of the sodium channel was decelerated. Taken together, these results provide electrophysiological evidence that TMCC prevents abnormal sodium currents induced by H/R by altering the steady-state inactivation kinetics.

In ventricular myocytes, the generation of action potentials is facilitated by the L-type Ca^{2+} channels under both physiological and pathophysiological conditions [21–23]. Our group has previously shown that TMCC increases $I_{Ca,L}$ activity, which has subsequent positive inotropic effects [12, 13]. In contrast, established anti-arrhythmia drugs operate through a separate mechanism, indicating that TMCC and established drugs will have non-overlapping effects. Our results showed that the H/R group significantly increased L-type calcium currents, and this effect was blunted by TMCC treatment. The H/R group shifted the steady-state activation curves to the left and shifted inactivation curves to the right, suggesting that the voltage-dependent steady-state activation of L-type Ca^{2+} channel was accelerated and inactivation of L-type Ca^{2+} channels was decelerated. These data suggested that the H/R group increased $I_{Ca,L}$ by facilitating of steady-state activation and inhibiting of steady-state inactivation. Both doses of TMCC or amiodarone restored abnormal L-type calcium currents by decreasing the effect of H/R. TMCC at the 400 μM dose showed effects similar to that of amiodarone. Following H/R injury, TMCC or amiodarone shifted the steady-state activation curves to the right and shifted the steady-state inactivation curve to the left, suggesting that the steady-state activation of the L-type calcium channel was decelerated and inactivation of the L-type calcium channel was accelerated. Our results provide electrophysiological evidence that TMCC prevents abnormal L-type calcium currents induced by H/R by inhibiting steady-state activation and facilitating steady-state inactivation.

I_{to} is the predominant repolarizing potassium current in rat ventricular myocytes [24]. Following depolarization, cardiac I_{to} channels activate and inactivate rapidly, and this response is necessary to maintain the resting membrane potential [24]. Depolarization is a key mechanism whereby ventricular arrhythmias in induced in pathophysiological setting, and preventing abnormal depolarization is the major target of Class III antiarrhythmic drugs [25]. Being able to inhibit I_{to} channels could potentially be therapeutically efficacious to prevent or suppress reentrant arrhythmias [26–28]. The result of the present study showed that transient outward potassium currents were significantly increased by H/R. The H/R group shifted steady-state activation to the left, while failed to alter the steady-state inactivation curves, suggesting that the steady-state activation of I_{to} channel was accelerated. These data suggested that the H/R group increased I_{to} through facilitation of steady-state activation. TMCC or amiodarone restored transient outward potassium currents significantly, which were increased by H/R. The effect of TMCC was equivalent to that of amiodarone. After H/R injury, TMCC and amiodarone shifted the steady-state activation curves to the right and failed to alter the steady-state inactivation curves, suggesting that the steady-state activation was decelerated. Our study provides electrophysiological evidence that TMCC prevents abnormal transient outward potassium cur-

rents induced by H/R by blocking steady-state activation at relatively higher concentrations. It is important to note that our study did not distinguish between the fast (I_{to},f) and slow (I_{to},s) components, and differences between these channels should be evaluated in future studies.

In conclusion, the results of this study demonstrate that TMCC can significantly relieve the effect of hypoxia/reoxygenation by modifying sodium, L-type calcium, and transient outward potassium channels. The predominant effect was to reverse the ionic balance in ventricular myocytes disrupted by H/R. Meanwhile, compared with amiodarone, the effect of TMCC prevented abnormal sodium currents induced by H/R was more potent than the effect of amiodarone. TMCC, accordingly, may be used as a multi-target antiarrhythmic drug in the future and a useful agent to prevent arrhythmias in the setting of ischemia and reperfusion.

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Conflict of Interest

None.

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