Laboratory Research

Ion mechanism of isoproterenol on delayed afterdepolarization and triggered activity in the infarcted ventricle

Jin-Liao Gao, Hong-Juan Wang, Yun-Feng Lan, Zhou Fang, Yan Liu, Min Lin, Yi-Cheng Fu, Yang Li

Institute of Geriatric Cardiology, Chinese PLA General Hospital Beijing 100853, China
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Objectives This study aimed at investigating the cellular mechanism of isoproterenol (ISO) on delayed afterdepolarizations (DADs) and triggered activity (TA) of the noninfarcted myocardium in the myocardial infarcted rabbit model.**Methods** Rabbits with the left anterior descending coronary artery occlusion were prepared and recovered for 8 wk (healed myocardial infarction, HMI). Myocytes were isolated from regions of the noninfarcted left ventricular free wall. ISO was added to cellular surface by perfusion way. Action potentials and ion currents were recorded with whole-cell patch clamp. **Results** The results showed that treatment with ISO induced more DADs and TA events in HMI myocytes. Iti and $I_{Ca\text{-}L}$ of myocytes treated with ISO were increased significantly compared with HMI cells, which contributed to DADs-related triggered arrhythmia. **Conclusions** The results suggested that more arrhythmia events of DADs and TA developed in myocytes with ISO treatment. The underlying mechanism was associated with the augment of I_{ti} and calcium influxing **(*J Geriatr Cardiol* 2010; 7:180-183)**.

Key words isoproterenol; healed myocardial infarction; delayed afterdepolarization; triggered activity; ion channels

Introduction

The noninfarcted myocardium in healed myocardial infarction (HMI) undergoes significant remodelling. Clinical and experimental studies have suggested that myocardial infarction is associated with a greater risk of sudden cardiac death (SCD), which is probably caused by lethal ventricular arrhythmias.[1,2] Although the cellular mechanisms of arrhythmogenesis in this pathophysiological condition are poorly understood, it may be explained that sympathetic nerve sprouting in HMI has been involved in augmented incidences of ventricular tachyarrhythmia.[3] Beta blockers, or drugs with beta blocking effects, are known to prevent these arrhythmias and SCD.[4,5] Several studies showed that numbers of premature contractions and the arrhythmias derived from the delayed afterdepolarizations (DADs) induces triggered activity (TA) in the noninfarcted myocardium after HMI.[6-8] We hypothesized that isoproterenol (ISO) mediated abnormal autorhythmicity arrhythmia after HMI. To prove this hypothesis, we examined the effects of ISO on DADs and TA in HMI model, and then investigated the ion channel mechanism.

Corresponding author: Yang Li, Institute of geriatric cardiology, Chinese PLA General Hospital, Beijing 100853, China; 10-6693-6762. Email: liyangbsh@ 163. com

Materials and methods

Model of myocardial infarction

A rabbit model of myocardial infarction was established as previously described.[9] New Zealand white rabbits (weighing between 2.0 to 2.5 kg) were anesthetized with pentobarbital (30 mg/kg, iv) and the left anterior descending coronary artery was ligated, and then the animals were allowed to recover for 4 wk, as the healed myocardial infarction group (n = 9, number of animals).

Isolation of ventricular myocytes

Rabbit ventricular myocytes were isolated as described previously.10 The heart was suspended on a Langendorff perfusion, and perfused with Tyrode's solution (in mmol/L) NaCl 135, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.33, HEPES 10 and glucose 10 (pH 7.4) containing 0.33 mg/mL collagenase, 0.025 mg/mL protease E and 1.25 mg/mL bovine serum albumin for 20 min. The tissue samples of the noninfarcted section in the left ventricular free wall were minced and sequentially digested for 20 to 25 min in fresh enzymes solution (36 ± 1℃). Cells in each region were stored, yielding to 60% - 80% single ventricular myocytes.

Isoproterenol treatment of ventricular myocytes

ISO was solved filtering water for store solution and diluted to final concentration of 0.1, 0.5, 1.0, 5.0 and 10 μM

with bath solution, which was added to the perfusion.

Patch clamp experiments

Quiescent, Ca-tolerant, rod-shaped cells with clear cross striation and a resting potential ≤ -80 mV were used for action potential recordings. Transmembrane potentials and currents were recorded by the whole cell patch-clamp technique using a MultiClamp 700B amplifier (Axon Instruments). All signals were acquired at 5 kHz (Digidata 1322A, Axon Instruments) and analyzed by pCLAMP version 9.2 software (Axon Instruments). Patch pipettes were pulled from borosilicate glass on a P-97 horizontal puller (Sutter Instruments). The electrodes had a resistance of 2-3MΩ.

Action potentials were elicited by using current-clamp mode at a rate of 5.0 Hz of 30 train suprathreshold current pulses. Myocytes were electrically stimulated by intracellular current injection through patch electrodes using depolarizing pulses with duration of 3 ms and amplitude of 1.5-2.5 nA. DADs are defined as a depolarization >5 mV for >10 ms occurring during diastole (phase 4) immediately after an action potential. TA is defined as an unstimulated action potential developing from a DAD[15].

Inward transient current (Iti) was induced by applying train pulses from -100 mV to + 30 mV for 2000 ms after 20 conditioned step pulses ranging from -80 to +50 mV in a duration of 150 ms. Pipettes were filled with (mmol/L): 120 K-aspartame acid, 4 Na_2ATP, 1 $MgCl_2$, 10 HEPES, 5 glucose, pH adjusted to 7.3 with KOH. Myocytes were perfused with solution containing (mmol/L): 140 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 4 KCl, 10 HEPES, 5 glucose, pH adjusted to 7.4 with NaOH. I_{ti} was measured as the difference between the peak of the "oscillation" and the basal membrane current. In case of successive I_{ti} oscillations, the first one was taken for analysis.

Extracellular solution with tetrodotoxin inhibit Na^+ currents and CsCl to inhibit K^+ currents was used. L-type calcium current (I_{Ca-L}) was recorded in voltage-clamp mode with 200 ms pulses from a holding potential of -40 mV, to different test potentials increased from -40 mV to +60 mV with 10 mV steps. Pipettes were filled with solution containing (mmol/L): 80 Cs-Aspartate acid, 10 NaCl, 2 $MgCl_2$, 40 CsCl, 10 EGTA, 2 Na_2ATP, 0.1 GTP, 10 HEPES, pH adjusted to 7.2 with CsOH. Myocytes were perfused with solution containing (mmol/L): 140 NaCl, 4 CsCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 5 glucose and 0.05 tetrodotoxin, pH adjusted to 7.4 with NaOH. Current-voltage (I-V) curves were obtained by applying voltage steps in 10 mV increments (-40 mV to +60 mV) for 150 ms from a holding potential of -40 mV. Steady-state activation (SSA) curves of I_{Ca-L} were determined by using pulses from -60 mV to +30 mV, 10 mV increments for 500 ms. Steady-state inactivation (SSI) curves of I_{Ca-L} were determined by using pulses from -70 mV to +30 mV, 10 mV increments for 1000 ms. SSA and SSI were described by assuming a Boltzmann function ($I/I_{max}=A1+A2/(1+\exp((V_{0.5}-V_{rev})/k)$. The time course of recovery from inactivation of I_{Ca-L} was evaluated with a paired-pulse protocol: conditioning pulse was applied to 0 mV from holding potential of -40 mV, following test potential of 0 mV for 150 ms after various interval duration of 0.1, 0.5, 1, 5, 10, 20, 40, 80, 160, 320, 640, 1280, 2560, 5120 ms. Recovery curve from inactivation was fitted by single exponential function.

Statistical analysis

Statistical analysis was performed using SPSS version 14. One way ANOVA with Bonferroni post hoc test or Student *t*-test was used for numerical data. Densitometric analysis was used for Western blot assays which retuned arbitrary units of band intensities. Chi square test (Fisher's exact test) was used to compare difference of incidence of DADs and TA. Data were expressed as mean ± S.E. $P<0.05$ was considered statistically significant.

Results

Effects of ISO on DADs and TA incidences

Our findings demonstrated an increased incidence of DADs in HMI myocytes, which was large enough to induce TA and arrhythmia in HMI. Furthermore, both DADs and TA events in myocytes with ISO were more frequently compared with HMI. DADs were elicited in 58.3% (7/12, number of cells) of myocytes with ISO 30 nM, significantly higher than that of the HMI (25.0 %, 3/12) (P <0.01, Figure 1 a,b and e). TA events were markedly increased to 50.0% (6/12) of myocytes with ISO 30 nM, compared with HMI myocytes (16.7%, 2/12, P <0.01, Figure 1, c,d and f).

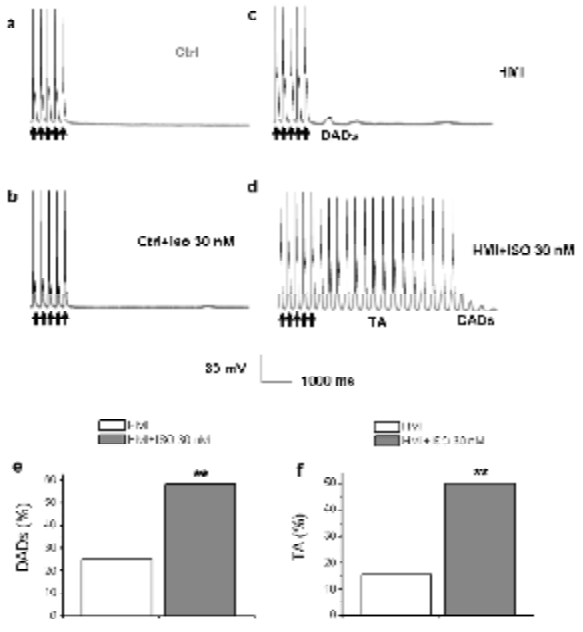

Fig. 1 Effect of ISO on DADs and TA in rabbit ventrcular myocytes at 5.0 Hz stimulation. Arrow indicated the last five paced action potnetials. DADs (a,b) or TA (c,d) was induced in HMI myocytes, more DADs and TA events in HMI myocytes with ISO 30 nM treatment were showed. The percents of DADs and TA events in myocytes ISO 1.0 μM were increased (e,f). $^{**}P$< 0.01 vs. HMI myocytes.

Effects of ISO on transient inward current (I_{ti})

The densities of I_{ti} were compared among three groups. Significant increase of I_{ti} was observed in myocytes with. At -50 mV of test potential, on average peak current densities of Iti increased from -0.7 ± 0.1 pA/pF in HMI to -2.3 ± 0.5 pA/pF in myocytes with ISO 30 nM (P<0.05, n=16, Figure 3, a). Current-voltage relation curves showed that density of inward I_{ti} was maximal at -50 mV. Comparing with HMI myocytes, the curves also presented that the current density of I_{ti} in myocytes with ISO 30 nM was significantly larger ranging from -90 mV to 0 mV (Figure 2, b).

Effects of ISO on calcium current (I_{Ca-L})

The mean current densities at 0 mV were -19.9 ± 1.2 pA/pF in myocytes with ISO 30 nM and -11.2 ± 1.3 pA/pF in HMI myocytes (P <0.01, n=15, Figure 3a). The current-voltage relations also showed that current densities in HMI myocytes with ISO 30 nM treatment were significantly increased range of -20 mV and +30 mV (Figure 3b). SSA curve of myocytes with ISO 30 nM was shifted to more negative. $V_{0.5}$,act was shifted from -21.6 ± 3.2 mV of HMI to -30.3 ± 2.9 mV of HMI with ISO 30 nM treatment (P <0.01, n=15) and kact values of activation of three groups were -9.9 ± 1.3 mV, -8.7 ± 2.3 mV and -10.3 ± 2.0 mV respectively. SSI curve in two groups had no significant difference (Figure 4, a). The recovered course from the inactivation of I_{Ca-L} in myocytes with ISO 30 nM was not significantly change (Figure 4, b).

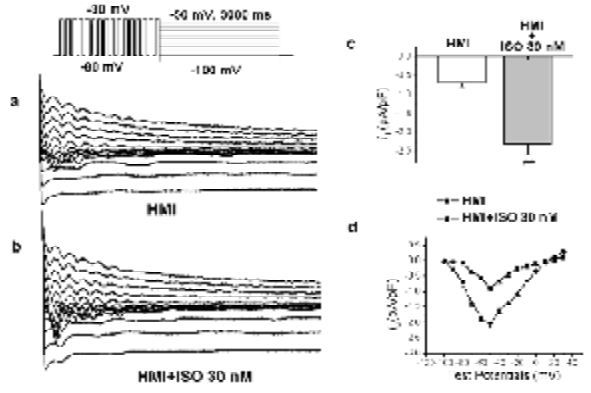

Fig. 2 Effect of ISO on Iti in rabbit ventrcular myocytes. Current amplitudes of HMI myocytes with ISO 30 nM treatment was larger than that of HMI myocytes (a). Current-voltage relationship showed that densities of inward Iti was maximal at -50 mV and decreased at more negative and more positive membrane potentials but densities of inward Iti of HMI myocytes with ISO 30 nM treatment was larger range of -90 mV and 0 mV (b). ** P < 0.01 vs. HMI myocytes.

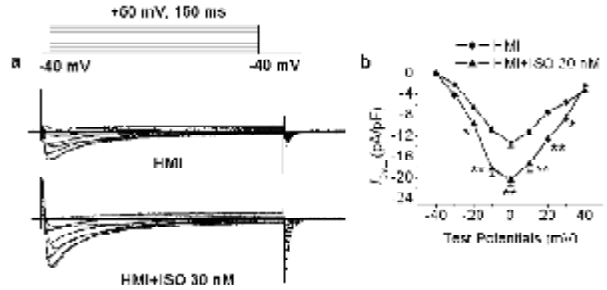

Fig. 3 Effect of ISO on I_{Ca-L} in rabbit ventrcular myocytes. Current amplitudes of HMI myocytes with ISO 30 nM treatment was significantly larger than that of HMI myocytes (a). The current-voltage relations also showed that current densities in HMI myocytes with ISO 30 nM treatment were significantly increased range of -20 mV and +30 mV (b).

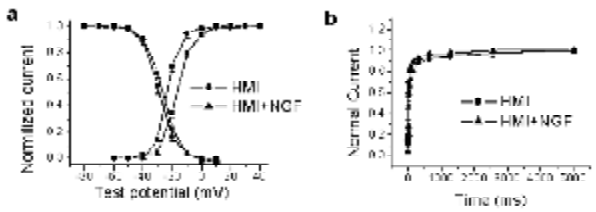

Fig. 4 Effect of ISO on gating mechanism of ICa-L in rabbit ventrcular myocytes. Steady-state activited curve of HMI myocytes with ISO 30 nM treatment was shifted to more negative potential, while steady-state inactivated curve in two groups had no significantly different (a). The time course of recovery from inactivation was well fitted by biexponential relation. A recovery from inactivation of ICa-L was change berfore and after expoure of ISO 30 nM (b).

Discussion

The autonomic nervous system represents a major regulatory mechanism of short-term and long-term adjustments of cardiac function via β -adrenergic receptors. In the heart, ¦Â-adrenergic receptors stimulated by activating the cAMP-dependent protein kinase (PKA), promotes the phosphorylation and activation of key components of the excitation-contraction coupling process. These include the sarcolemmal L-type Ca^{2+} channels, which are responsible for the initial Ca^{2+} influx; the ryanodine receptors, which allow Ca^{2+} release from the sarcoplasmic reticulum.

This study showed that the cellular electrophysiological was changed by ISO which was infused to rabbit HMI ventricular myocytes. Significantly, ISO treatment increases DADs and TA incidences. Abnormal initiation resulted in either automaticity or TA with a DADs attained threshold potential.[11,12] Cao and coworkers[13,14] provided evidence implicating nerve sprouting in ventricular arrhythmogenesis and potentially SCD. Their observations suggested an association between post-injury sympathetic nerve density and susceptibility to life-threatening ventricular arrhythmias in these patients. Based on the effect of ISO to prolong the

action potential plateau of myocardium of HMI myocytes suggested a greater effect of ISO to increase intracellular Ca^{2+} in myocardium from afflicted than normal animals, it is likely that the abnormal signal transduction in the afflicted group is associated with excess Ca^{2+} that generates the occurrence of DADs. Collectively, this evidence indicated that ISO caused the nerve remodeling was likely to play significant roles in the increased risk of life-threatening arrhythmias after MI.

The DADs during ventricular tachycardia is involved closely in I_{ti}.[15] I_{ti} is believed to be the current that is responsible for the generation of oscillatory after potentials which is a underlying mechanism of abnormal automaticity involved in a variety of cardiac arrhythmias. In this article, I_{ti} increased significantly in HMI myocytes with ISO infusion. It was indicated that the increase of I_{ti} in noninfarction region myocytes was likely a major factor contributing to DADs and TA. The mechanism might involve stimulating β-adrenergic receptors, openning of activated $[Ca^{2+}]i$-activated nonselective cation current, $[Ca^{2+}]i$ activated chloride current, and/or the electrogenic $Na+/Ca^{2+}$ exchange and induces I_{ti}.[16] The extra Ca^{2+} influx through L-type Ca^{2+} channels at the prolonged plateau phase can induce more Ca^{2+} release from SR stores[17] and lead to intracellular Ca^{2+} overload, a condition known to enhance the triggering of DADs.[18] The transient inward current (I_{ti}) forms the ionic basis for generation of DADs.[19] Increased density of sympathetic nerve could augment release and cause higher than normal tissue concentrations of sympathetic neurotransmitters during sympathetic excitation. Spatially heterogeneous electrical remodeling of cardiomyocytes might result in an increase of L-type Ca^{2+} current density, causing action potential prolongation in hyperinnervated regions. Intracellular calcium overload could give rise to TA and VT/VF. Liu et al [20] demonstrated in rabbits that hypercholesterolemia induces proarrhythmic neural and myocardial remodeling. We found that ISO, a sympathetic nervation agonist, notably increased the densities of $I_{Ca\text{-}L}$ with fast activated procedure and larger window current and short time constant of recovery from inactivation. In the HMI myocytes, ISO can stimulate adenylyl cyclase activity and increase the level of the second messenger cAMP that activates PKA. Outcome of this well-defined signaling pathway in ventricular myocytes is to increase Ca^{2+} influx through phosphorylation-activated L-type Ca^{2+} channels.[21] The combination of pacing with ISO-stimulation is an established experimental protocol used to produce Ca^{2+} overload and trigger DADs, which mimics disruption of Ca^{2+} homeostasis under pathological conditions such as MI. [22] The larger $I_{Ca\text{-}L}$ played a vital role in producing I_{ti},[23,24] while expected to offset the reduced eject properties of the compromised infarcted heart, can lead to delayed afterdepolarization arrhythmias.[25]

Conclusions

In this study, we found that rabbit ventricular cells with ISO treatment after HMI increases abnormal automaticity or triggered activity caused with the delayed afterdepolarizations, which involved in the calcium influxing through the L-type channels and enhancing Iti. This effect play a important role of creating spontaneous VT,VF, and SCD.

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