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Experimental paper

Effect and mechanism of esmolol given during cardiopulmonary resuscitation in a porcine ventricular fibrillation model☆

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ABSTRACT

Objectives: The aim of the study was to investigate the effect on calcium cycling protein and electrical restitution of β_1-adrenergic receptor antagonist esmolol administered during cardiopulmonary resuscitation in the porcine ventricular fibrillation model.
Methods: Ventricular fibrillation untreated for four minutes was induced by dynamic steady state pacing protocol in 40 healthy male pigs, in which local unipolar electrograms were recorded using one 10-electrode catheter that was sutured to the left ventricular epicardium. During CPR, animals were randomized into two groups to receive saline as placebo or esmolol after two standard doses of epinephrine. At post-resuscitation 2-h, six pigs were randomly selected from each group and the second VF induction was performed. Local activation-recovery intervals (ARI) restitutions and the VF inducibility between control group and esmolol group were compared. Western blotting was performed to determine expression of Ca^{2+}/calmodulin-dependent protein kinase IIδ(CaMKIIδ) and cardiac ryanodine receptor (RyR2) protein, and their phosphorylation status.
Results: Injection of esmolol combined with epinephrine during CPR significantly decreased recurrent rate of ventricular fibrillation during 2-h post-resuscitation, meanwhile it has no adverse affect on the restore of spontaneous circulation. Esmolol significantly flattened ARI restitution slope, lessened regional difference of ARI restitution, decreased the VF inducibility, and alleviated CaMKIIδ hyper-activation and RyR2 hyper-phosphorylation.
Conclusions: Esmolol given during CPR has significant effects on modulating electrical restitution property and intracellular calcium handling, which contributes the most important reasons why β_1-blockade significantly reduced the onset and maintenance of VF.

1. Introduction

Previous findings[1–4] suggest that β-adrenergic antagonist may deserve consideration as a therapeutic intervention during advanced life support for prolonged ventricular fibrillation on the basis that β-blockade can attenuate potentially dangerous hyperadrenergic state and improve myocardial bioenergetics. Recently, action potential duration (APD) restitution properties and repolarization alternans are thought to be important arrhythmogenic factors.[5–7] Unbalance of intracellular calcium homeostasis and APD alternans act synergistically, as well as with tissue heterogeneity, to promote wavebreak and fibrillation.[5,8–10] Furthermore, it is important to note that key Ca^{2+} signaling molecules such as Ca^{2+}/calmodulin-dependent protein kinase II δ (CaMKIIδ) is emerging as focal points for studying calcium homeostasis, ventricular arrhythmias, and heart dysfunction in cardiac diseases.[11–14] We hypothesized that effects of esmolol given during CPR be closely linked to modulate APD restitution property, the phosphorylation status of calcium cycling protein, especially in the context of hyperadrenergic state during VF, CPR and the early post-resuscitation period.

2. Methods

Animals used in this study received humane care in compliance with the principles of laboratory animal care.

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2.1. Animal preparation

Forty healthy male Yorkshire-cross domestic pigs weighing between 25 and 30 kg were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg), and maintained intravenously by additional doses of 1 mg/kg of sodium pentobarbital. After endotracheal intubation, animals were mechanically ventilated with a tidal volume of 15 mL/kg with the aid of a volume-controlled ventilator equipped with an infrared mainstream analyzer (model PM-7000, Mindray Bio-Medical Electronics Corporation, ShenZhen, China) for the monitor PET_{CO2}. Respiratory frequency was adjusted to maintain PET_{CO2} between 35 and 40 mmHg.

For measurement of aortic pressure, a fluid-filled arterial catheter (6F, Medtronic Inc., Minneapolis, USA) was advanced from the femoral artery into the descending thoracic aorta. Mean arterial pressure (MAP) was determined by the electronic integration of the aortic blood pressure waveform. For the determination of coronary perfusion pressure (CPP), a Swan-Ganz catheter (5F, Arrow International Inc., USA) was advanced from the internal jugular vein into the right atrium. CPP was calculated as the difference between aortic diastolic pressure and the simultaneously measured right atrial diastolic pressure. For the measurement of left ventricular function, the right carotid artery was cannulated by an arterial catheter with a microtip pressure transducer for the measurements of left ventricular diastolic end pressures (LVEDP), $LV + dp/d_{max}$ (max rate of pressure rise) and $LV - dp/dt_{max}$ (max rate of pressure decay) during baseline, cardiac arrest, restore of spontaneous circulation (ROSC), and 2 h after ventricular fibrillation, meanwhile body temperature and surface ECG were monitored. Aforementioned hemodynamic measurements were recorded by the electrophysiological analyzer (64-lead, Prucka Engineering Inc., Houston, Texas, USA).

2.2. Experimental procedure

The heart was exposed by a median sternotomy, and soaked in warm (37 °C) physiological saline intermittently to prevent surface cooling. Local unipolar electrograms (UEs) were recorded using one 10-electrode catheter (2-8-2 mm, 6F, Biosense Webster, Inc., Diamond Bar, CA, USA). The catheter was sutured to the left ventricular epicardium (Fig. 1). A stainless steel needle was inserted into the chest wall subcutaneously as the reference for the mapping electrodes. In the catheter, the 1st, 3rd, 5th, 7th and 9th electrode served as the exploring electrodes, with a distance of 11 mm to the next. Unipolar electrograms were recorded by the Prucka 64-lead electrophysiological analyzer with a sampling rate of 800 Hz, and were displayed at 100 mm/s screen speed and 10 mm/mV amplitude. A 0.05-to 500-Hz bandpass filter was applied to obtain high quality unipolar signals.

A protocol of dynamic steady state pacing (SSP) was applied to induce VF as in previous study [6,15,16]. The ventricle was paced at a pacing strength of twice the diastolic threshold via a Teflon-coated bipolar silver needle electrode (2-ms pulse duration) at the epicardium of the right ventricular out-flows tract. The basic cycle length determined the initial pacing interval (PI). Each pulse train was delivered and maintained for no less than 60 s before next pacing train. Then PI was progressively shortened in 10-ms steps for cycle length of more than 200 ms, and in 5 ms steps for cycle length of less than 200 ms until 1:1 capture was lost. Pacing strength was progressively elevated (by steps of 1 v) to regain the 1:1 capture. The dynamic decrease of PI was performed until VF was induced. After induction of VF, mechanical ventilation was stopped.

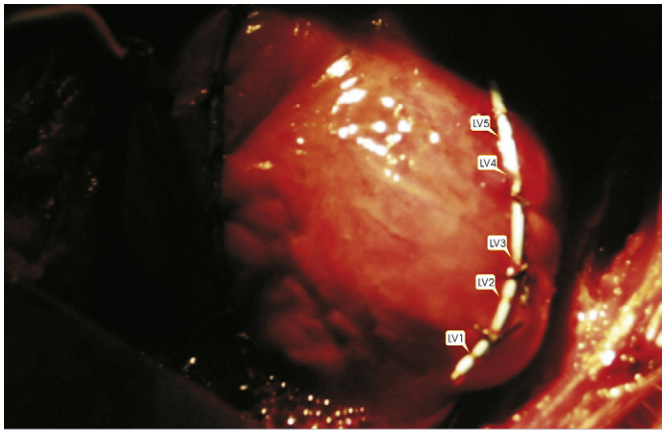

Fig. 1. Contact mapping was performed in five sites in the LV epicardium. Local unipolar electrograms (UEs) were recorded using one 10-electrode catheter that was sutured to the left ventricular epicardium. The 1st electrode of 10-electrode catheter was fixed to the apex (LV1), the 5th electrode (LV3) was approach to LV anterior wall, and the 9th electrode (LV5) was attached to the base near outflow tract.

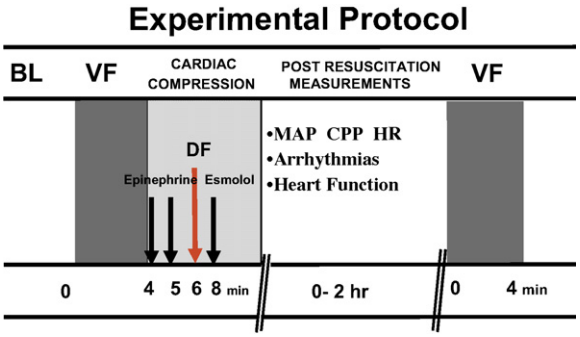

Fig. 2. Diagram of protocol of the first ventricular fibrillation induced by dynamic steady state pacing (SSP) stimulation, cardiopulmonary resuscitation, measurements durings first 2 h after VF, and the second VF induced by SSP stimulation. DF indicates defibrillation.

The experimental procedure of cardiopulmonary resuscitation was performed as follow (Fig. 2). Mechanical ventilation and uninterrupted cardiac compression were initiated after 4 min of untreated VF. Each animal was given intravenously two standard doses of epinephrine (20 μg kg^{-1}, every bolus). About 2 min later,

Table 1
The maximum slope for local ARI restitution curves ($\bar{x} \pm S$, $N = 6$).

	LV1(Red)	LV2(Green)	LV3(Blue)	LV4(Black)	LV5 (Yellow)
Normal heart	1.75 ± 0.03	1.38 ± 0.02	1.29 ± 0.03	1.49 ± 0.02	2.31 ± 0.03
Control group	1.80 ± 0.04	2.26 ± 0.03	1.62 ± 0.03	2.62 ± 0.03	3.31 ± 0.04
Es group	1.32 ± 0.03*	1.33 ± 0.03*	1.16 ± 0.02*	1.28 ± 0.03*	1.60 ± 0.02*
T value	16.6277	53.6951	31.2500	77.3672	93.6601
P value	0.0001	0.0001	0.0001	0.0001	0.0001

LV1 (Red) was the 1st electrode of 10-electrode catheter which was fixed to the apex; LV3 (Blue) was the 5th electrode of 10-electrode catheter which was approach to LV anterior wall; LV5 (Yellow) was the 9th electrode of 10-electrode catheter which was attached to the base near outflow tract.

* $P < 0.05$ vs Control group.

one defibrillation shock 20-J was given (M series Biphasic Option, Zoll, USA). If VF was not reversed after one shock, the animals were randomized to receiving saline as placebo (20 mL dilution, bolus) or Esmolol (0.5 mg/kg per 20 mL dilution, bolus). The CPR operators were blinded to the treatment, and only the principal investigator, who did not take part in any resuscitation effort, knew the assignment of each animal. Furthermore, the investigators involved in data recording and analysis were also blinded to the allocation. Cardiac compression was continued between multiple shocks. Uninterrupted CPR duty was performed in those animals with post-shock asystole or pulseless electrical activity until spontaneous organized cardiac rhythm. If ROSC was associated with systolic arterial pressure of less than 60 mmHg, dopamine was infused ($50\,\mu g\,kg^{-1}\,min^{-1}$) and maintained. Resuscitation was considered

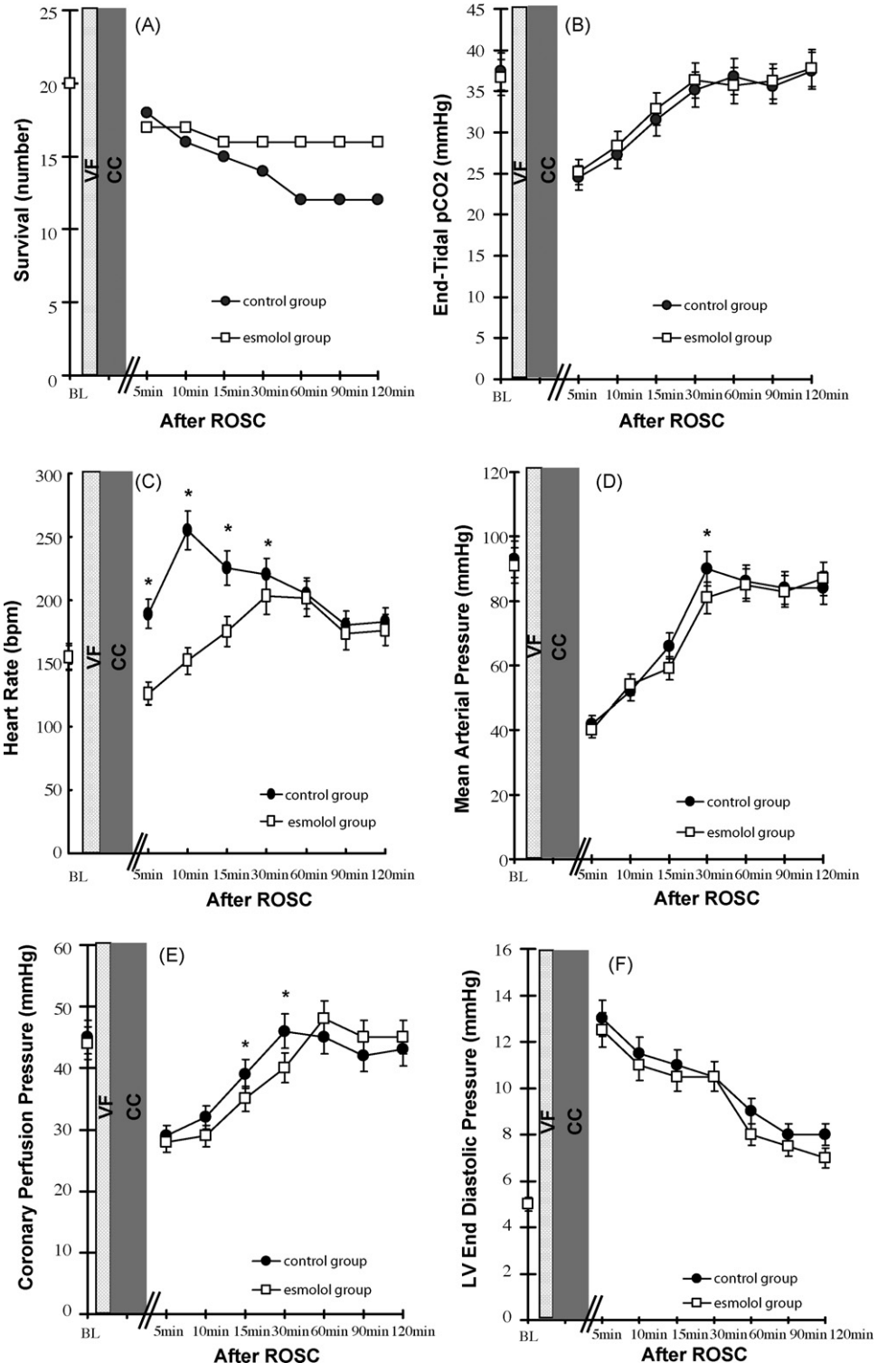

Fig. 3. Comparison of survival numbers, $PETCO_2$, HR, CPP, MAP, and myocardial performance during 2 h post-resuscitation between control group and esmolol group. Close circles represent animals received epinephrine during CPR. Open squares represent animals received epinephrine combined with esmolol during CPR. VF indicates ventricular fibrillation, CC indicates cardiac compression, and ROSC indicates restore of spontaneous circulation. $^{*}P < 0.05$ *vs* Esmolol group.

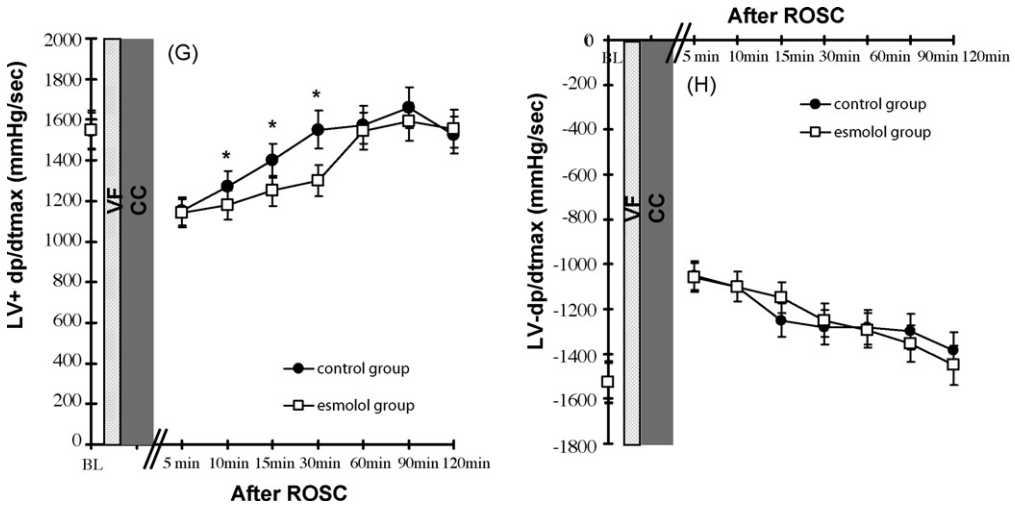

Fig. 3. (*Continued*).

successful when an organized cardiac rhythm, accompanied by a MAP of at least 60 mmHg, for a minimum of five minutes. The animal was pronounced dead when resuscitation remained unsuccessful at the end of 30 min CPR attempt. At the post-resuscitation 2-h, six successfully resuscitated pigs were randomly select from each group and applied to another SSP protocol. The study was ended after the second VF induction was maintained for four minutes.

2.3. Signal processing and construction of restitution curves

Local UEs was replayed at 200 mm/s screen speed and 20 mm/mV amplitude on the Prucka 64-lead electrophysiological analyzer. Wave signals between the initial PI and the shortest PI were analyzed. Signals without a smooth and stable diastolic baseline were excluded from analysis. At least five stable waves in each pulse train were employed and averaged to minimize measurement error.

Activation-recovery intervals (ARIs) for local UEs represent the time course of repolarization of the intracellular action potential. ARI was defined as the interval between activation time and repolarization time. Diastolic interval (DI) was defined as the period between the end of repolarization time of the preceding beat and the activation time of the following beat. Restitution curves were constructed by plotting ARI versus DI for local UE recording (Origin, Microcal Software). An exponential curve (Eq. (1)) using the Levenberg-Marquardt method was used for this analysis[6,15]:

$$y = a - \beta e^{-x/\tau} \quad (1)$$

With each β and τ, and DI, the slope was constructed using Eq. (2):

$$\text{slope} = \frac{\beta}{\tau * e^{-x/\tau}} \quad (2)$$

The maximal slope (Smax) of the restitution curve was defined as the slope of the shortest DI of the restitution curve. The R^2 is a quantitative value for the degree of fitness of the model. The maximum pacing interval for inducing VF was served as a quantitative measure of VF inducibility.

2.4. Western blotting analysis

After pericardiotomy, a weight of 100 mg myocytes of the LV base from each animal was subjected to Western blotting, using primary antibodies to CaMKIIδ (1:500, Santa Cruz Biotechnology), phospho-CaMKIIδ (1:500, Affinity BioReagents), RyR2 (1:200, Santa Cruz Biotechnology), rabbit polyclonal antibodies against phosphorylated RyR2-Ser^{2815} or RyR2-Ser^{2809} (Genesil Biotechnology, Wuhan, China), and GAPDH (1:500, Santa Cruz Biotechnology), used as a loading control. The bands were quantified by densitometric analysis (Gel Pro 4.5, USA). The intensity of aforementioned bands was normalized to GAPDH from identical tissue as a control. The investigator performed western blotting analysis was also blinded to the experimental groups.

2.5. Statistical analysis

Data are expressed as mean and standard deviation. Student's *t* test was used for the comparison of different time measurement of hemodynamic parameters for each group and comparison of local ARI restitution curve slopes with normal distribution. Comparison of post-resuscitation 2-h survival rate and recurrence rate of VF between groups were performed a Chi-Square analysis. Comparison of percentage about semi-quantitative analysis of calcium cycling protein expression and phosphorylation status between groups was analyzed using the Kruskal-Wallis test. A P value ≤ 0.05 was considered as statistically significant. All analyses were conducted using the SPSS software (version 13.00).

3. Results

3.1. Comparison of resuscitation characteristics, post-resuscitation outcome variables between the two groups

None of the forty animals restored spontaneous circulation after initial defibrillation attempts. Seventeen out of the twenty animals in the esmolol group and eighteen out of the twenty animals in the control group were successfully resuscitated. There was no significant difference in the mean CPR time of survivors between two groups (13 ± 2.5 *vs* 12 ± 2.8 min, $t = 1.11$, $p = 0.31$). Six animals in the control group and seven animals in the esmolol group required dopamine support. However, there were a greater number of shocks other than the first rescue shock being delivered during CPR in the control group compared with the esmolol group (3.5 ± 0.5 *vs* 1.5 ± 0.5, $t = 11.76$, $p = 0.0001$). After 2 h, sixteen of 17 animals survived in the esmolol group, and one of them died of circulatory collapse; whereas twelve of 18 animals survived in the control group, five animals died of VF and one died of heart failure. Lower

recurrence of ventricular fibrillation within post-resuscitation 2-h was significantly observed in the esmolol group (0/17 *vs* 5/18, $p = 0.0001$). Compared with injection of epinephrine alone, relatively higher survival numbers during post-resuscitation 2-h were observed in the esmolol group, although no significant improvement was shown in short-term survival rate (16/20 *vs* 12/20, $p = 0.168$).

Compared with the control group, a significantly lower HR, MAP, CPP, and peak $\pm dP/dt$ of the left ventricular pressure trace happened within 30 min post-resuscitation in the esmolol group (Fig. 3C–E, G), while LVEDP and peak $-dP/dt$ of the LV pressure trace were not different between the two groups (Fig. 3F and H).

3.2. Local ARI restitutions

In the first SSP stimulation, we observed that regional difference of ARI restitution exists in the normal ventricle. The slopes of the ARI restitution curve at the LV apex and LV base were significantly steeper than those of LV anterior wall. During the second SSP stimulation, ARI restitution slope maximum of the control group at the LV anterior wall raised significantly from 1.29 ± 0.03 to 1.62 ± 0.03 and at the LV base from 2.31 ± 0.03 to 3.31 ± 0.04 (Table 1). Meanwhile, ARI restitution slope maximum of the esmolol group significantly flattened; for instance, those of LV base descended to 1.60 ± 0.02, but it also has a visible effect on the modulating regional difference of ARI restitution (Fig. 4A–C).

Pacing intervals for inducing VF were 130–190 ms (mean, 161 ± 18 ms, $N = 40$) in the first SSP stimulation. During the second SSP stimulation, pacing intervals for inducing VF were significantly greater in the control group compared with the esmolol group (263 ± 12 *vs* 218 ± 11 ms, $N = 6$, $t = 6.77$, $p = 0.001$).

3.3. CaMKIIδ protein expression and phosphorylation status

Although CaMKIIδ protein expression normalized to GAPDH demonstrated no statistical significance between groups, phosphorylated CaMKIIδ (phosphorylated CaMKIIδ/GAPDH) was high at the myocardium of death animals in the control group, lower survivors within post-resuscitation 2-h in the control group and lowest survival pigs in the esmolol group (2.91 ± 0.05, 2.28 ± 0.03, 1.32 ± 0.03, $N = 6$, respectively, Fig. 5A–C and G). Phosphorylated ratio of CaMKIIδ (phosphorylated CaMKIIδ/CaMKIIδ) was high at the myocardium of death animals in the control group, lower survival pigs in the control group and lowest survival pigs in the esmolol group (0.91 ± 0.05, 0.83 ± 0.03, 0.46 ± 0.03, $N = 6$, respectively, Fig. 5A–C, G).

3.4. RyR2 protein expression and phosphorylation status

Although RyR2 protein expression normalized to GAPDH demonstrated no statistical significance, phosphorylated RyR2 at Ser^{2815} site (RyR2-P^{2815}/GAPDH) was high at the myocardium of death animals in the control group, lower in survivors within post-resuscitation 2-h in the control group and lowest in survival pigs in the esmolol group (1.76 ± 0.05, 0.88 ± 0.03, 0.46 ± 0.03, $N = 6$, respectively, Fig. 5D–F, H). Also, the phosphorylated RyR2 at Ser^{2809} site (RyR2-P^{2809}/GAPDH) have varied between groups (1.20 ± 0.04, 0.58 ± 0.03, 0.60 ± 0.03, $N = 6$, respectively, Fig. 5D–F, H). Furthermore, the phosphorylated ratios of RyR2 at both Ser^{2815} (RyR2-P^{2815}/RyR2) and Ser^{2809} sites (RyR2-P^{2809}/RyR2) have varied remarkably between groups (death animals in the control group *vs* survivors in the control group *vs* esmolol group, 0.67 ± 0.05 *vs* 0.35 ± 0.02 *vs* 0.17 ± 0.02 for Ser^{2815} site, 0.46 ± 0.04 *vs* 0.23 ± 0.03 *vs* 0.21 ± 0.03 for Ser^{2809} site, Fig. 5D–F, H, $N = 6$).

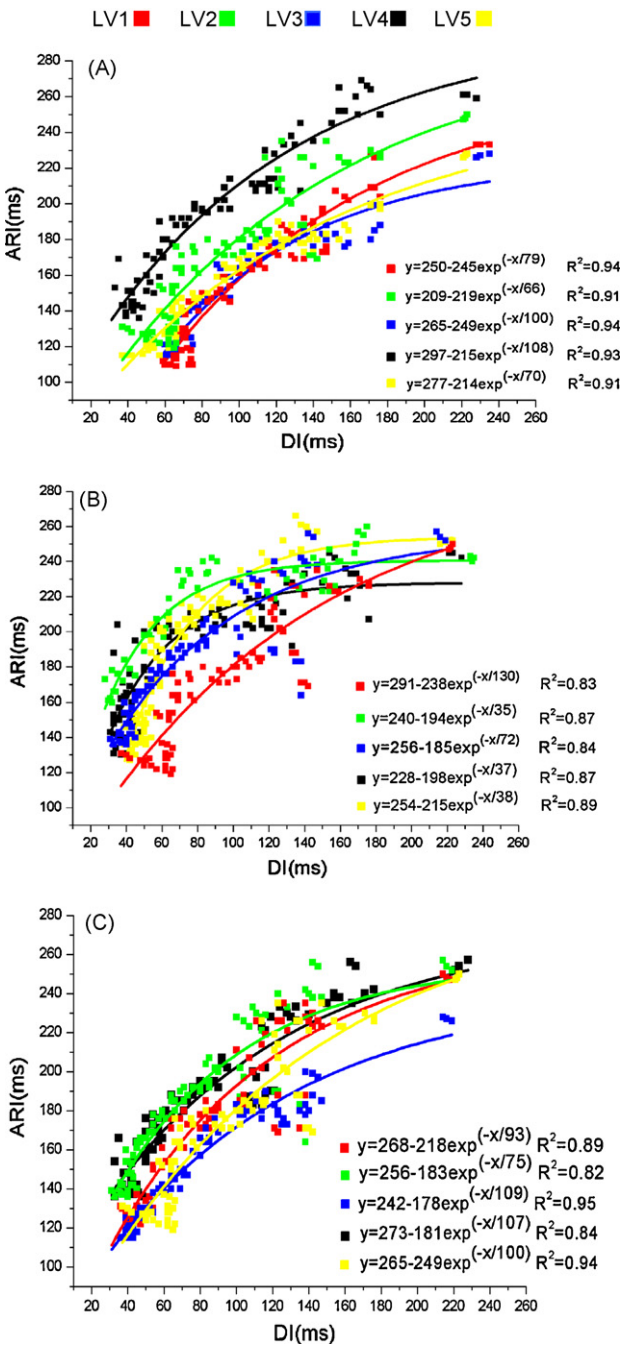

Fig. 4. Activation-recovery intervals (ARI) restitution curves ($N = 6$). (A) ARI restitution curves of 5 mapping electrodes from left ventricular apex to base near during the first SSP stimulation. (B) ARI restitution curves of 5 different sites during second SSP stimulation in animals received epinephrine. (C) ARI restitution curves of 5 different sites during second SSP stimulation in animals received epinephrine combined with esmolol.

4. Discussion

4.1. Main finding

The major findings of this study are as follows: (1) Injection of esmolol combined with epinephrine during CPR significantly decreased recurrent rate of ventricular fibrillation during 2-h post-resuscitation, meanwhile it has no adverse affect on the restore of spontaneous circulation; (2) Higher phosphorylation status of CaMKIIδ and RyR2 were observed at the myocardium of the animals received epinephrine alone. Esmolol contributed to alleviate

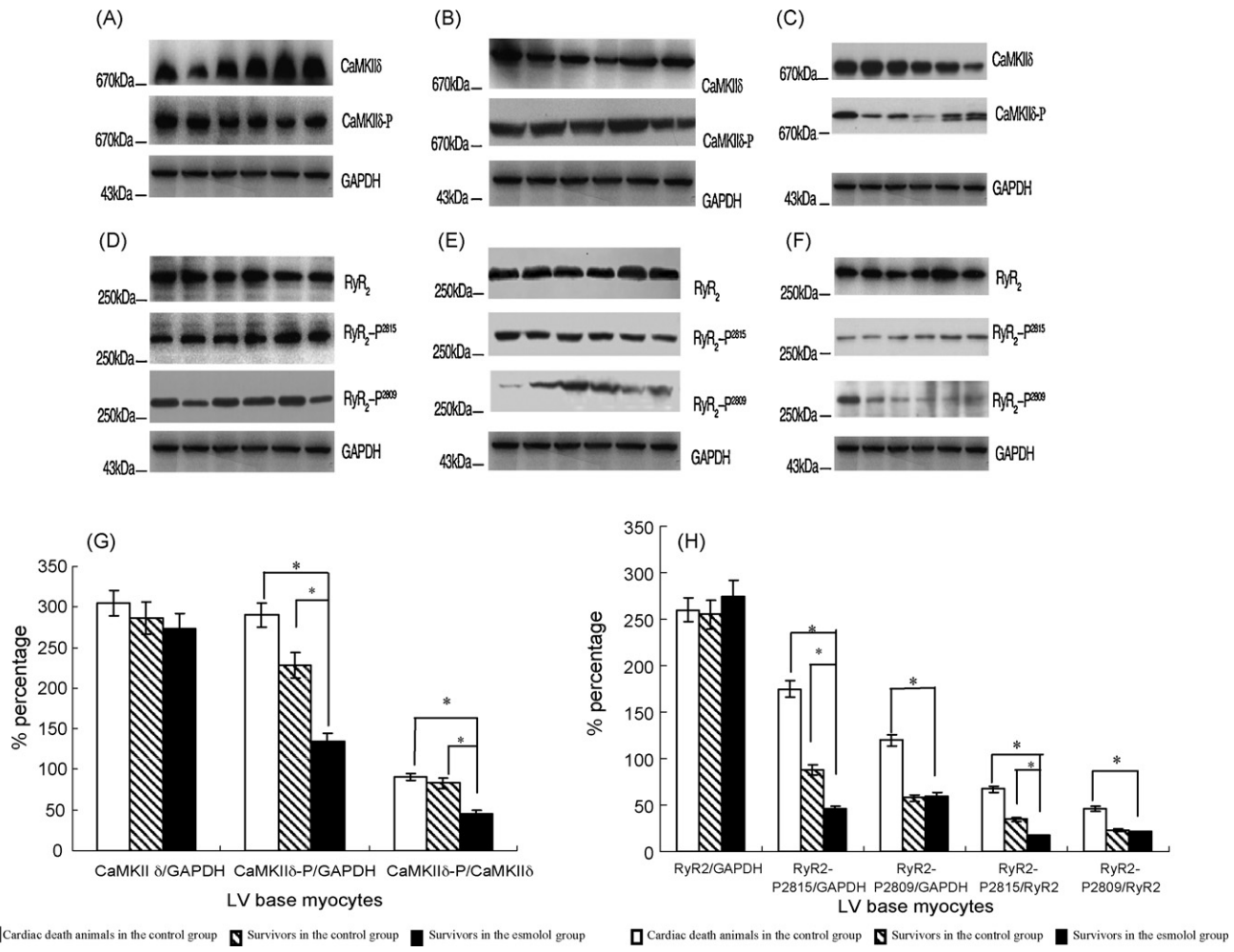

Fig. 5. CaMKIIδ and RyR2 protein expression and phosphorylation status. A, B and C demonstrate western blot of CaMKIIδ, phosphorylated CaMKIIδ, and GAPDH from LV tissue of death animals in the control group, survivors within post-resuscitation 2-h in the control group, and survivors in the esmolol group, respectively. $N = 6$. D, E and F demonstrate western blot of RyR2, phosphorylated RyR2 at CaMKIIδ-dependent site (Ser^{2815}) and PKA- dependent site (Ser^{2809}), and GAPDH from LV tissue of death animals in the control group, survivors within post-resuscitation 2-h in the control group, and survivors in the esmolol group, respectively. $N = 6$. G. CaMKIIδ relative protein expression and phosphorylated ratio of CaMKIIδ between groups; H. RyR2, phosphorylated RyR2 at Ser^{2815} and Ser^{2809} sites relative protein expression, and phosphorylated ratio of RyR2 at Ser^{2815} and Ser^{2809} sites between groups. $^{*}P < 0.05$ vs Esmolol group.

their hyper-phosphorylation; (3) The effect of epinephrine was to steepen ARI restitution curve, as well as to aggravate spatial heterogeneity of ARI restitution property in the whole heart. Esmolol significantly blocked epinephrine's effect on the electrical restitution.

4.2. The effect of epinephrine and esmolol on electrical restitution property

In the present study, a significantly smaller number of electrical shocks were required in the esmolol group during CPR. Furthermore, esmolol dramatically decreased the VF inducibility. Our findings support that these benefits of esmolol, as a β_1-adrenergic receptor antagonist, due to blocking the effects on electrical restitution property of catecholamines, significantly flattened the ARI restitution slope and lessened regional difference of ARI restitution. Spatial heterogeneity of electrical restitution property, as do steep ARI restitution curve, may play an important role in the VF maintenance mechanism. If the ARI restitution curve has a steep slope (ARI restitution slope > 1), then ARI is sensitive to small changes in DI. In other words, these wavelength oscillations are self-amplifying. If regional difference is also present (e.g., wavelength oscillations in the A site are not identical those in the B site), then the wavelength oscillations are remarkably unstable, and the rotor breaks up spontaneously into multiple wavelets as a result of dynamically induced functional heterogeneities.[5,17–23]

4.3. The effect of epinephrine and esmolol on intracellular calcium cycling protein

Recent findings support that CaMKIIδ is a key downstream effector of the β_1-adrenergic receptor signaling pathways, and CaMKIIδ is regarded as a molecular determinant for maintaining calcium homeostasis in normal and diseased cardiac myocytes.[24–29] Wehrens and Kohlhaas showed that this was most likely attributable to increased CaMKIIδ-dependent RyR2 phosphorylation at Ser^{2815} site enhancing RyR openings, independent of PKA; because Ca^{2+} sparks frequency could be reduced back to normal levels by blocking CaMKIIδ.[12,30–32] Furthermore, Sarcoplasmic reticulum (SR) calcium cycling is increasingly recognized as a critical determinant of APD slopes and APD alternans. Once spontaneous SR calcium is released, delayed afterdepolarizations is arising through calcium-sensitive ionic currents, including the L-type calcium current, the Na-Ca exchange current, and calcium-activated nonselective and chloride currents. This triggered activity, which happened on the 4th phase of APD, can drive APD to alter-

nate secondarily. In short, delayed afterdepolarizations arising from CaMKIIδ-dependent RyR phosphorylation contributes to aberrant RyR openings, augmented electrical instability, triggered arrhythmias, and sudden cardiac death.

Our studies revealed that the highest ratio of phosphorylated RyR2 both at Ser^{2815} site and Ser^{2809} site existed at the myocardium of cardiac death animals receiving epinephrine alone. Furthermore, the phosphorylated ratios of RyR2 at PKA-dependent site (Ser^{2809}) demonstrated no statistical significance between survivors in the control group and those in the esmolol group, while higher ratio of phosphorylated RyR2 at CaMKIIδ- dependent site (Ser^{2815}) existed at the myocardium of survivors in the control group compared with those in the esmolol group. It is noted that esmolol given at the early stage of CPR is effective in suppressing RyR2 hyper-phosphorylation with regard to PKA phosphorylation of Ser^{2809} and CaMKIIδ phosphorylation of Ser^{2815}. We speculated that aforementioned effect of esmolol is one of the most important mechanisms in reducing fatal ventricular arrhythmias and cardiac death.

4.4. The effect of esmolol on hemodynamic effect and myocardial performance

In our study, esmolol blunted transiently and reversibly the hemodynamic response including heart rate, MAP on the early stage of ROSC, which could not provide adverse effect on the percentage of survivors, mean CPR time, and 2-h post-resuscitation myocardial performance. This finding is in accordance with the findings of Killingsworth,[1] because esmolol is a short-acting β_1-blockade with a half-life of 9 min, whose period of action was similar to the duration of the catecholamine surge.

However, it was unclear how direct and precise effects of acute CaMKIIδ hyper-phosphorylation upon β_1-AR activation alter the long-time post-resuscitation heart function. CaMKIIδ-dependent RyR2 phosphorylation increases RyR2 open probability, enhances diastolic SR calcium leak, reduces SR calcium content, and possibly deteriorates cardiac systolic function. At the same time, upon phosphorylation of phospholamban by CaMKIIδ, the inhibition of SERCA is removed, allowing for regulated uptake of cytosolic Ca^{2+} back into the SR. It was therefore logical to assume that mechanisms of esmolol's effect on the heart function are complex and multifactorial.

It is unclear that CaMKIIδ effects may be major or sole mediators of post-resuscitation heart function because β_1-blockade given during CPR affects myocytes performance extensively. Firstly, esmolol helps to balance oxygen demand and oxygen consumption when epinephrine increases myocardial work as well as oxygen consumption.[33–36] Secondly, our study showed that esmolol reduced mean number of electrical shock.

Tang indicated that[37] less shock defibrillation protocol significantly improved heart outcome and survival, because the less shock was associated with reducing CPR interruptions of total resuscitation time, mitigating electrical shock damage to myocytes.

5. Limitation

Firstly, conclusion of the present study was based on the limited number of recordings from five sites located on the LV epicardium. Secondly, monitoring local UEs of experimental animals and post-resuscitation arrhythmias were on the anesthetized state, and potentially obfuscating effects of the anesthetic agents were not excluded.

6. Conclusion

Esmolol given during CPR significantly flatten ARI restitution slope, lessen regional difference of ARI restitution, decreased the VF inducibility, and alleviate CaMKIIδ hyper-activation and RyR2 hyper-phosphorylation, which effects make up the most important reasons why β_1-blockade significantly reduced the onset and maintenance of VF.

Conflict of interest

There is no conflict of interest.

Acknowledgments

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