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IVABRADINE-INDUCED HEART RATE REDUCTION INCREASES THE SEVERITY OF POSTRESUSCITATION MYOCARDIAL DYSFUNCTION IN A RAT MODEL OF CARDIOPULMONARY RESUSCITATION

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ABSTRACT—**Aims:** A rapid heart rate (HR) that occurs after cardiopulmonary resuscitation (CPR) is a short-term compensatory mechanism preserving cardiac output. However, if of long duration, it is unfavorable for myocardial function postresuscitation because of disrupted balance between myocardial oxygen supply and demand. This raises the assumption that such a sustained fast HR should be regulated. The present study aimed to investigate the follow-on effect of ivabradine (a specific inhibitor of the I_f current of the sinoatrial node)–induced HR reduction (HRR) on postresuscitation myocardial function in a rat model of CPR. **Methods and results:** Six minutes of ventricular fibrillation and 8 min of CPR were performed on Sprague-Dawley rats. All 32 resuscitated animals were then randomized into saline and ivabradine groups, each group having nonsurvival and survival subgroups (n = 8 each). Saline or ivabradine (0.5 mL/kg) was administered at 1 h postresuscitation. Heart rate, myocardial function as expressed by cardiac output, ejection fraction, and myocardial performance index were assessed at baseline and hourly from 1 to 5 h postresuscitation. Heart rate variability was analyzed at baseline and at 1, 3, and 5 h postresuscitation. Serum epinephrine and cardiac troponin I at baseline and at 1, 3, and 5 h postresuscitation in nonsurvival subgroup were measured. Survival duration in the survival subgroup was observed. The baseline HR was approximately 390 beats/min (bpm). After resuscitation, an average increase of $\Delta \approx +15$ bpm (relative ratio ≈ +3.8%) with a resultant HR of 405 bpm lasting more than 5 h occurred. Ivabradine group achieved a steady HRR of $\Delta \approx -30$ bpm (relative ratio ≈ –7.4%) as compared with saline group ($P < 0.01$). Postresuscitation myocardial function was significantly worse in the ivabradine group (all $P < 0.01$). Heart rate variability was significantly impaired in the ivabradine group (all $P < 0.05$). Serum cardiac troponin I and epinephrine concentration were significantly higher in the ivabradine group (all $P < 0.01$). Survival duration was significantly shortened in the ivabradine group as compared with the saline group (388 vs. 526 min, $P < 0.01$). **Conclusions:** Ivabradine-induced HRR increases the severity of postresuscitation myocardial dysfunction and shortens survival duration in a rat model of CPR.

KEYWORDS—Cardiopulmonary resuscitation, heart rate, ivabradine, myocardial function, postresuscitation

ABBREVIATIONS—CA–cardiac arrest, CO–cardiac output, CPR–cardiopulmonary resuscitation, EF–ejection fraction, $ETCO_2$–end-tidal carbon dioxide, HR–heart rate, HRR–heart rate reduction, LVEDV–left ventricle end-diastolic volume, LVFT–left ventricular filling time, MAP–mean arterial pressure, MFI–microvascular flow index, MPI–myocardial performance index, PVD–perfused vessel density, ROSC–return of spontaneous circulation, RRI–R-R interval, SVR–systemic vascular resistance, SV–stroke volume, TTM–targeted temperature management

INTRODUCTION

Postresuscitation (PR) myocardial dysfunction, one key component of post–cardiac arrest syndrome, is responsible for low survival rates after cardiac arrest (CA) (1). The severity of PR myocardial dysfunction, a key factor in early PR death, is now known to correlate with the duration of ischemia (2,3), the number of electrical defibrillations, and administration of epinephrine during cardiopulmonary resuscitation (CPR) (3).

Heart rate (HR), an intuitive vital sign, is closely allied to myocardial dysfunction (4). On the one hand, a rapid HR as a result of sympathetic hyperactivity is a compensatory mechanism preserving the cardiac output (CO) when myocardial function is depressed. On the other hand, if of long duration, the fast HR increases myocardial oxygen demand, shortens diastole, and reduces myocardial oxygen supply, thus aggravating myocardial dysfunction (5). After CA and CPR, a long-lasting rapid HR occurs after return of spontaneous circulation (ROSC). An earlier study illustrated that in a rat model of CPR, 4 and 8 min of ventricular fibrillation plus 8 min of CPR, respectively, resulted in steady HRs of 415 and 440 beats/min (bpm) at 1 h PR lasting more than 2 to 3 h as compared with a baseline HR of 360 bpm (3), leading to an

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The study protocol was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University (no. AD10001396). All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health.

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imbalance between oxygen supply and demand unfavorable for PR myocardial function. This then leads to the assumption that reduction in such HR should be beneficial.

Among all the HR-reducing agents, ivabradine (IVA) provides a specific HR reduction (HRR) by inhibiting the I_f current of the sinoatrial node (6). This agent having no negative inotropic effect has been approved by guidelines for treating heart failure with reduced ejection fraction (EF). Animal studies also illustrate several favorable effects of IVA-induced HRR on exercise-induced myocardial ischemia and stunning, energy metabolism, and septic microcirculatory derangements.

In the present study, we aimed to investigate the follow-on effect of IVA-induced HRR on PR myocardial function in a rat model of CPR. It is hypothesized that with those proven beneficial effects, IVA-induced HRR would also improve PR myocardial dysfunction and prolong duration of survival.

MATERIALS AND METHODS

Animals

Healthy male Sprague-Dawley rats, aged 6 to 8 months, weighing between 450 and 550 g, were supplied by a single-source breeder (Envigo Laboratories, Frederick, MD), which has consistently supplied healthy animals of relatively uniform age and weight. The study protocol was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University (no. AD10001396). All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health.

Chemical

Ivabradine hydrochloride (SML0281; Sigma-Aldrich, Burlington, MA) 10 mg was dissolved in 10 mL saline (SAL) (0.9% NaCl). The final solution (1 mg/mL) IVA hydrochloride was kept at 4°C.

Rat model of CPR

The rats were housed in a quiet environment with 12-h day/night cycles and had free access to water and food except for being fasted overnight before the experiment.

After induction of anesthesia with inhalation of CO_2 for 30 s, animals were anesthetized by i.p. injection of pentobarbital (45 mg/kg). Additional doses of pentobarbital (10 mg/kg) were administered at intervals of 1 h or when required to maintain anesthesia. Anesthetized animals were placed on an operating surface (Hard Plastic Water Circulating Operating Board; Texas Scientific Instruments, Fair Oaks Ranch, TX) connected to a heating/warming machine (InnerCool STx Surface Pad System; Zoll Medical Corporation, Pittsburgh, PA). A conventional lead II electrocardiogram was continuously monitored. The trachea was orally intubated with a 14-gauge cannula mounted on a blunt needle (Abbocath-T; Abbott Hospital Products Division, North Chicago, IL) with a 145-degree angled tip. End-tidal carbon dioxide ($ETCO_2$) was continuously monitored with a sidestream infrared CO_2 analyzer (Capstar-100 Carbon Dioxide Analyzer; CWE Incorporation, Ardmore, PA) interposed between the tracheal cannula and ventilator. Animals breathed room air spontaneously during preparation. A polyethylene catheter (PE-50; Becton-Dickinson and Company, Franklin Lakes, NJ) was advanced from the left femoral artery into the descending aorta for the measurement of arterial pressure and serum biomarkers. A thermocouple microprobe (IT-18; Physitemp Instruments Incorporation, Clifton, NJ) was inserted into the left femoral vein and advanced into the inferior vena cava to measure blood temperature. An additional PE-50 catheter was advanced through the left external jugular vein and into the right atrium for the measurement of right atrial pressure. Aortic and right atrial pressures were measured with high-sensitivity transducers (Model 42,584-01; Abbott Critical Care Systems, North Chicago, IL). A 3F catheter (Model C-PMS-301 J; Cook Critical Care, Bloomington, IN) was advanced through the right external jugular vein and into the right atrium. A precurved guide wire was then advanced through the catheter and into the right ventricle to induce ventricular fibrillation. All catheters were flushed intermittently with SAL containing 2.5 IU/mL of crystalline bovine heparin. Blood temperature during the experiment was maintained at 36.5°C to 37.5°C by a water-circulating heating system.

Fifteen minutes before inducing ventricular fibrillation, baseline hemodynamic measurements and echocardiography were obtained. Mechanical ventilation was established at a tidal volume of 0.60 mL/100 g of body weight, a frequency of 100 breaths/min, and an inspired O_2 fraction of 0.21. Ventricular fibrillation was then induced through a guide wire advanced from the right jugular vein into the right ventricle. A 2.0- to 2.5-mA, 60-Hz current was then delivered to the right ventricular endocardium. The current was continued for 3 min to prevent spontaneous defibrillation. Mechanical ventilation was discontinued after the onset of ventricular fibrillation. After 6 min of untreated ventricular fibrillation, being 6 min of CA, precordial chest compression (a rate of 200 bpm with equal compression and relaxation) was given in an anteroposterior position using a custom-made pneumatically driven and electronically controlled piston device (Supplementary Material S1, http://links.lww.com/SHK/B551), together with mechanical ventilation (tidal volume, 0.60 mL/100 g body weight; frequency, 100 breaths/min; O_2 fraction, 1.0) was initiated for a duration of 8 min. The depth of compressions was initially adjusted to maintain a coronary perfusion pressure of 22 ± 2 mm Hg. After precordial compression, an electrical shock with 4-J was attempted after 8 min of CPR. Return of spontaneous circulation was defined as the ROSC with a mean aortic pressure greater than 50 mm Hg for 5 min. If ROSC was not achieved after the first defibrillation attempt, a 30-s interval of precordial chest compressions was performed before the next defibrillation attempt (up to three attempts). After resuscitation, mechanical ventilation was continued with O_2 fraction of 1.0 for 1 h, O_2 fraction of 0.5 for the following hour, and then O_2 fraction of 0.21 for 3 h.

Experimental design

Ten minutes after successful resuscitation, animals were randomized into two groups (n = 16 for each group): (1) SAL (untreated HR following resuscitation) and (2) IVA (lowered HR after resuscitation) with 0.9% NaCl (0.5 mL/kg) and IVA (0.5 mL/kg) solution were respectively administered *via* the left external jugular vein catheter immediately after echocardiography and microcirculation measurement or blood draw at PR 60 min (PR 60′). Each group was further divided into two subgroups: (i) survival and (ii) nonsurvival (n = 8 for each subgroup). Our pilot study had demonstrated that 2.0, 1.0, and 0.5 mL/kg IVA, respectively, resulted in survival durations of 60, 90, and 300 min as compared with a natural survival duration of 500 min; 0.5 mL/kg was a sufficient dose to illustrate the differences giving long enough for observation (Supplementary Material S2, http://links.lww.com/SHK/B552). Rats in the nonsurvival subgroup had a 0.5 mL blood draw from the left femoral artery catheter at baseline and at PR 60′, 180′, and 300′, followed by euthanasia. In the survival subgroup, all catheters including the endotracheal tube were removed at PR 300′ when the animals had recovered from anesthesia. Animals were given an s.c. injection of buprenorphine SR LAB (0.5 mg/mL; ZooPharm) (1 mg/kg) on the back of the neck for analgesia and then returned to their cages and closely observed.

Measurements

Electrocardiogram, aortic and right atrial pressures, $ETCO_2$, and blood temperature values were continuously recorded on a personal computer–based data-acquisition system supported by WINDAQ software (DATAQ Instruments Incorporation, Akron, OH). Coronary perfusion pressure was calculated as the difference between diastolic aortic and time-coincident right atrial pressure measured at the end of each minute of precordial compression in real time. Defibrillation times and total doses of pentobarbital were also recorded.

HR, MAP, and $ETCO_2$

Heart rate was computed using the R-R interval (RRI) on 5-min stable electrocardiogram. MAP was calculated using the following formula: MAP = diastolic arterial pressure + 1/3 × (systolic arterial pressure − diastolic arterial pressure). The readings of HR, MAP, and $ETCO_2$ at baseline; at PR 60′, 65′, 75′, and 90′; and hourly from PR 120′ to 300′ were selected.

Echocardiographic variables and systemic vascular resistance

Doppler and two-dimensional echocardiographic examinations were performed using a Philips system (HD 11 XE; Philips Medical Systems, Bothell, WA) with a 12.5-MHz transducer at baseline and hourly from PR 60′ to 300′. Left ventricular filling time (LVFT) was calculated as the sum of isovolumetric relaxation time and mitral inflow time using a derivative method from Tei et al. (7) (Fig. 1). Left ventricle end-diastolic volume (LVEDV) was measured using the area-length method. Stroke volume (SV) was measured using the left ventricular outflow tract velocity time integral (LVOT-VTI) method. Ejection fraction was calculated as the SV/LVEDV. Cardiac output was calculated as the SV × HR. Myocardial performance index was calculated according to the formula $(a - b)/b$, where a = mitral valve closure-to-opening interval and b = left ventricular ejection time. All images were analyzed by three blinded independent investigators. Systemic vascular resistance (SVR) was computed using the formula SVR = (MAP − RAP)/CO (8).

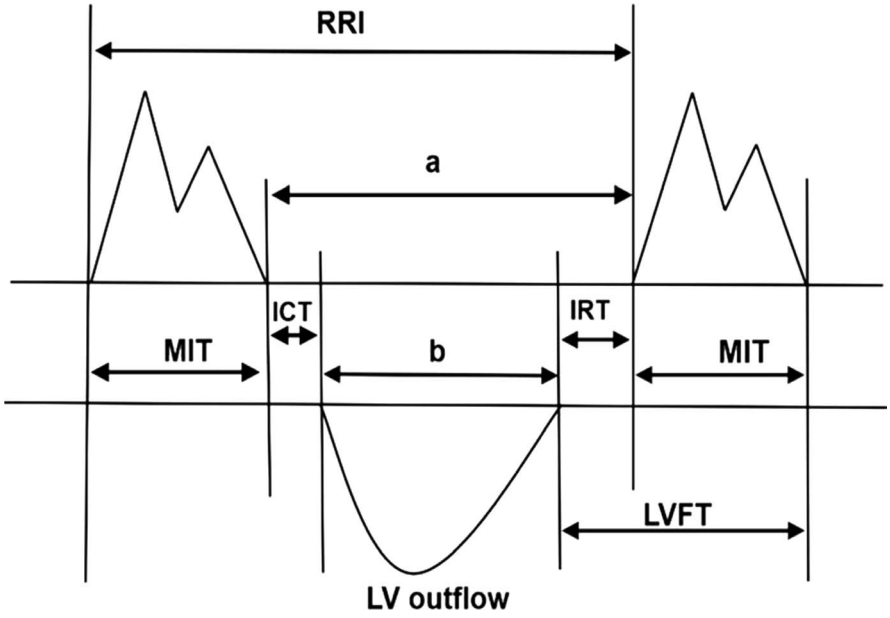

FIG. 1. **A schema using a derivative method from Tei et al. (7) illustrates measurement of Doppler intervals.** a is the interval between cessation and onset of the mitral inflow; b is the left ventricular ejection time. ICT, isovolumic contraction time; IRT, isovolumic relaxation time; LVFT, left ventricle filling time (=IRT + MIT); MIT, mitral inflow time; RRI, R-R interval.

HR variability

Heart rate variability (HRV) analyses were performed at baseline and PR 60′, 180′, and 300′ by the software HRVanalysis 1.0 (ANS Lab Tools, Université Jean Monnet, Saint-Etienne, France) (9) using the electrocardiogram data imported from WINDAQ software to determine frequency-domain indexes including low-frequency power (LF, 0.25–0.75 Hz) for sympathetic tone, high-frequency power (HF, 0.75–2.5 Hz) for parasympathetic tone, and the ratio of low- to high-frequency power (LF/HF) (10).

Buccal microcirculation

Buccal microcirculation was measured at baseline and hourly from PR 60′ to 300′ with the aid of a sidestream dark-field imaging device (MicroScan; Micro Vision Medical Incorporation, Amsterdam, the Netherlands). Microvascular flow index (MFI) was quantified by Spronk and colleagues' (11) method. The image was divided into four quadrants, and the predominant flow type (absent = 0, intermittent = 1, sluggish = 2, normal = 3) was assessed in the small vessels of each quadrant, which were less than 20 μm in diameter. Perfused vessel density (PVD) was measured based on De Backer and colleagues' (12) method. Vessel density was calculated as the number of vessels crossing the lines divided by the total length of the lines. All recordings were analyzed by three blinded independent investigators.

Serum concentrations of cardiac troponin I and epinephrine

For all nonsurvival animals, 0.5 mL of blood was withdrawn for analysis of cardiac troponin I (cTnI) and epinephrine, and then 0.5 mL of SAL was administered at baseline and PR 60′, 180′, and 300′. The serum concentrations of cTnI and epinephrine were measured with commercial enzyme-linked immunosorbent assay kits (2010-2-HSP [Life Diagnostics, Inc., West Chester, PA, USA] for cTnI, CSB-E08677h [Cusabio Biotech Co., Ltd., China] for epinephrine).

Survival duration

For all survival animals, survival duration was recorded in minutes.

Data analysis and statistical calculations

Normal distribution of all measured data was confirmed with Kolmogorov-Smirnov test. All measurements were reported as the mean ± SD. Comparisons of all measurements between groups were performed with two-way ANOVA. Comparisons within groups were performed with ANOVA repeated measurement with Bonferroni *post hoc* test. Two-tailed Student *t* test was used for two-group comparison in survival duration. $P < 0.05$ was deemed significant.

RESULTS

A total of 43 rats were utilized. Eleven animals were excluded (six rats had poor baseline data, and five rats were not resuscitated). There was no difference in baseline measurements (Table 1), parameters during CPR (Table 2), and blood temperature at any time point (Fig. 2) between the two groups.

CPR resulted in a long-duration rapid HR PR and IVA administration induced HRR

The baseline HR was approximately 390 bpm. After CA and CPR, an average increase of $\Delta \approx +15$ bpm (relative ratio ≈ +3.8%) with a resultant HR of approximately 405 bpm lasting more than 5 h occurred, as was shown in the SAL group. A single dose of IVA 0.5 mg/kg at PR 60′ achieved a sustained and steady HRR of $\Delta \approx -30$ bpm from PR 65′ to 300′ (all $P < 0.01$ except $P < 0.05$ at PR 65′), which was a very small relative decline ($\Delta \approx -7.4\%$) (Fig. 3A).

IVA-induced HRR was associated with increased MAP and decreased $ETCO_2$

Following resuscitation, MAP fell at first, then increased, and finally remained at a stable level in both groups. After IVA administration, MAP further declined and then rose from PR 75′ to PR 120′ (all $P < 0.05$). It then stayed at a higher level than the SAL group from PR 120′ to PR 300′ (all $P < 0.05$) (Fig. 3A). When compared with pre-CA levels, $ETCO_2$ levels were decreased after resuscitation. However, they dropped at a faster speed in the IVA group with lower values at each time point from PR 75′ to PR 300′ (PR 75′–90′, all $P < 0.05$; PR 120′–300′, all $P < 0.01$) (Fig. 3A).

IVA-induced HRR caused increases in RRI, LVFT, and LVEDV while decreases in SV

R-R interval and LVFT were significantly decreased after resuscitation in both groups compared with baseline, and then lower values were maintained in the untreated fast HR group. However, both parameters in the IVA group were significantly increased from PR 120′ to PR 300′ (for RRI, all $P < 0.05$; for

LVFT, all $P < 0.01$) (Fig. 3B). In both groups, LVEDV was significantly increased after resuscitation from PR 60′ to PR 300′ in both groups. However, this parameter increased at a faster speed in the IVA group at every time point from PR 120′ to 300′ (all $P < 0.01$) (Fig. 3B). The reverse situation was seen with SV (all $P < 0.01$) (Fig. 3B).

IVA-induced HRR increased the severity of myocardial dysfunction PR

For both groups, PR myocardial function as expressed by CO, EF, and myocardial performance index was impaired compared with baseline. However, the severity of PR myocardial dysfunction was significantly greater in the IVA group from PR 120′ to 300′ (all $P < 0.01$) (Fig. 3C). The IVA group showed higher SVR from PR 120′ to 300′ (all $P < 0.01$) (Fig. 3C).

IVA-induced HRR led to altered HRV and serum epinephrine indicative of sympathetic hyperactivity

As compared with the SAL group, the IVA group exhibited significantly increased LF and LF/HF but decreased HF from PR 120′ to 300′ (all $P < 0.01$) (Fig. 4, A–C). After resuscitation, serum epinephrine level did not differ between the groups at baseline and PR 60′. However, greater increases in serum epinephrine levels were observed in the IVA group at PR 180′ and 300′ (all $P < 0.01$) (Fig. 4D). All of those changes indicated significantly higher sympathetic tone.

IVA-induced HRR deteriorated microcirculation derangement

Buccal microcirculation as measured by PVD and MFI was reduced after resuscitation in both groups compared with baseline. Greater impairment in buccal microcirculation was observed in the IVA group from PR 120′ to 300′ (all $P < 0.01$) (Fig. 5).

TABLE 1. **Baseline characteristics**

Baseline characteristics		
Group	SAL (n = 16)	IVA (n = 16)
Body weight, g	511 ± 19	506 ± 22
Blood temperature, °C	37.1 ± 0.20	37.0 ± 0.18
HR, beats/min	390 ± 22	388 ± 25
MAP, mm Hg	140 ± 15	142 ± 17
RAP, mm Hg	1.8 ± 0.24	1.9 ± 0.18
$ETCO_2$, mm Hg	40 ± 1.9	41 ± 2.3
RRI, ms	155 ± 9.5	155 ± 11.0
LVFT, ms	98 ± 6.7	98 ± 7.4
LVEDV, μL	309 ± 13	312 ± 14
SV, μL	233 ± 12	240 ± 9
CO, mL/min	91 ± 9.6	93 ± 9.0
EF, %	75 ± 0.6	77 ± 0.9
MPI	0.62 ± 0.07	0.61 ± 0.06
SVR, min × mm Hg/mL	1.55 ± 0.20	1.53 ± 0.23
PVD	6.25 ± 4.49	6.33 ± 4.49
MFI	3 ± 0	3 ± 0
TDP, mg	29 ± 3.3	27 ± 2.2

Mean ± SD values are shown. The figure indicates there was no difference in baseline characteristics between the two groups.
CO indicates cardiac output; EF, ejection fraction; $ETCO_2$, end-tidal carbon dioxide; HR, heart rate; LVEDV, left ventricle end-diastolic volume; LVFT, left ventricular filling time; MFI, microvascular flow index; MPI, myocardial performance index; PVD, perfused vessel density; RAP, right atrial pressure; RRI, R-R interval; SV, stroke volume; SVR, systemic vascular resistance; TDP, total dose of pentobarbital within from start to 300 min postresuscitation.

TABLE 2. **Parameters during cardiopulmonary resuscitation**

Parameters during cardiopulmonary resuscitation		
Group	SAL (n = 16)	IVA (n = 16)
CPP at PC1, mm Hg	25 ± 2.7	26 ± 2.1
PC5, mm Hg	26 ± 2.6	25 ± 2.0
PC7, mm Hg	26 ± 1.6	26 ± 1.0
$ETCO_2$ at PC1, mm Hg	16 ± 2.2	18 ± 2.7
PC5, mm Hg	13 ± 2.2	14 ± 2.3
PC7, mm Hg	13 ± 2.5	13 ± 1.8
Defibrillation times	1.4 ± 0.5	1.6 ± 0.5

Mean ± SD values are shown. The figure indicates there was no difference in parameters during cardiopulmonary resuscitation between the two groups.
CPP indicates coronary perfusion pressure; $ETCO_2$, end-tidal carbon dioxide; PC1, 1 min after precordial compression; PC5, 5 min after precordial compression; PC7, 7 min after precordial compression.

IVA-induced HRR aggravated myocardial insult and shortened survival duration

After resuscitation, serum concentrations of cTnI did not differ between the groups at baseline and PR 60′. However, it was greater in the IVA group than the SAL group at PR 180′ and 300′ (all $P < 0.01$) (Fig. 6A). The SAL group showed an average survival duration of 526 min. The IVA group showed a significantly shortened average duration of survival (388 min) ($\Delta \approx -138$ min) ($P < 0.01$) (Fig. 6B).

DISCUSSION

In the present study, 6 min of ischemia and 8 min of reperfusion with low coronary perfusion pressure during CPR resulted in PR myocardial dysfunction and a rapid HR faster than baseline level with a duration of more than 5 h after ROSC. It further demonstrated that 0.5 mg/kg IVA administered at 1 h PR achieved a very slight degree of HRR after ROSC with strikingly increased sympathetic tone, severity of PR myocardial dysfunction, and shortened survival duration.

By contrast, in another porcine model of CPR, a beneficial follow-on consequence of IVA-induced HRR showed that infusion of IVA resulted in an HRR of 30 to 50 bpm after ROSC with improved PR myocardial function (13). The main reason for the divergence lies in the administered epinephrine during CPR in the porcine model, which helps to improve ROSC rate but results in worse PR myocardial function associated with a "significantly greater HR" (3). It is plausible that IVA improves PR myocardial function by neutralizing additional HR acceleration induced by administered epinephrine through activation of I_f current (14). Although the porcine model of epinephrine-assisted CPR is closer to a human setting, the established rat model of CPR has a unique advantage that it eliminates interference from exogenous epinephrine and also provides cardiopulmonary measurements comparable to those in vertebrates and human patients (3).

Cardiac output, the product of SV and HR, is an indicator of systemic blood flow. A decrease in SV, by activating the sympathetic nervous system, leads to increased HR to stabilize CO. In this study, a reduced SV due to global stunning of the myocardium following systemic I/R occurred and brought about a 5-h rapid HR after ROSC. It helped to preserve CO at the cost of

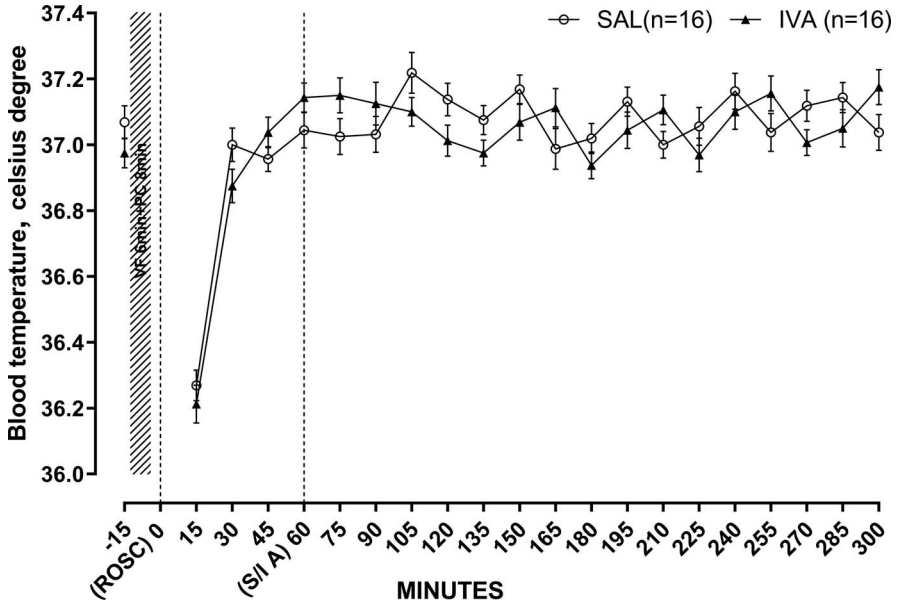

FIG. 2. **Plots show blood temperatures in both groups**. IVA, ivabradine; n, n minutes postresuscitation; ROSC, return of spontaneous circulation; SAL, saline placebo; S/I A, saline/ivabradine administration.

disrupting the balance between myocardial oxygen demand and supply (15,16). The gradual degeneration of serum CTnI and PR myocardial function in our investigation were at least in part attributed to this mechanism.

Slowing HR, by prolonging LVFT, leads to an increase in LVEDV (preload). Using Frank-Starling's law, an increased LVEDV stretches the cardiac muscle fibers, resulting in enhanced myocardium contractility and elevated SV. According to this mechanism, IVA-induced HRR leads to slow HR with increased SV and therefore unchanged CO followed by optimized oxygen supply/demand ratio (17), which is the basis of those beneficial effects aforementioned. The present study demonstrated that IVA-induced HRR yielded a longer RRI and LVFT, allowing an extended LVEDV. However, this LVEDV extension failed to translate into an increase in SV. A reasonable interpretation for this failure is that the global stunned myocardium cannot respond to the Frank-Starling mechanism. It is based on earlier findings that the failing heart is not able to use the Frank-Starling law because of myofibrillar calcium desensitization after an increase of the sarcomere length (18), which is involved in the I/R-induced stunned myocardium (19). Therefore, because of this failure of "stretch sensitization" (20) in PR myocardium, after IVA administration, the slow HR with an SV that failed to increase must have resulted in significant decreases in CO, EF followed by sympathetic hyperactivity as confirmed by the markedly altered HRV, and greater epinephrine production.

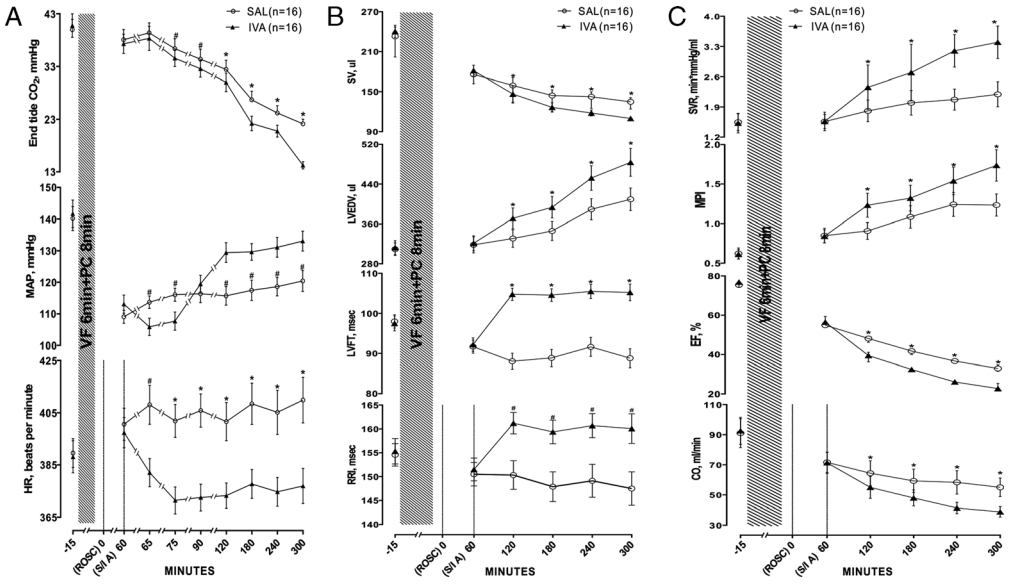

FIG. 3. **Scatterplots show the effect of ivabradine on heart rate, hemodynamics, and myocardial function (A–C), microcirculation (D)**. #$P < 0.05$, *$P < 0.01$ versus IVA group. –15, baseline; CO, cardiac output; EF, ejection fraction; $ETCO_2$, end-tidal carbon dioxide; HR, heart rate; IVA, ivabradine; LVEDV, left ventricle end-diastolic volume; LVFT, left ventricular filling time; MPI, myocardial performance index; n, n minutes postresuscitation; RAP, right atrial pressure; ROSC, return of spontaneous circulation; RRI, R-R interval; S/I A, saline/ivabradine administration; SAL, saline placebo; SV, stroke volume; SVR, systemic vascular resistance.

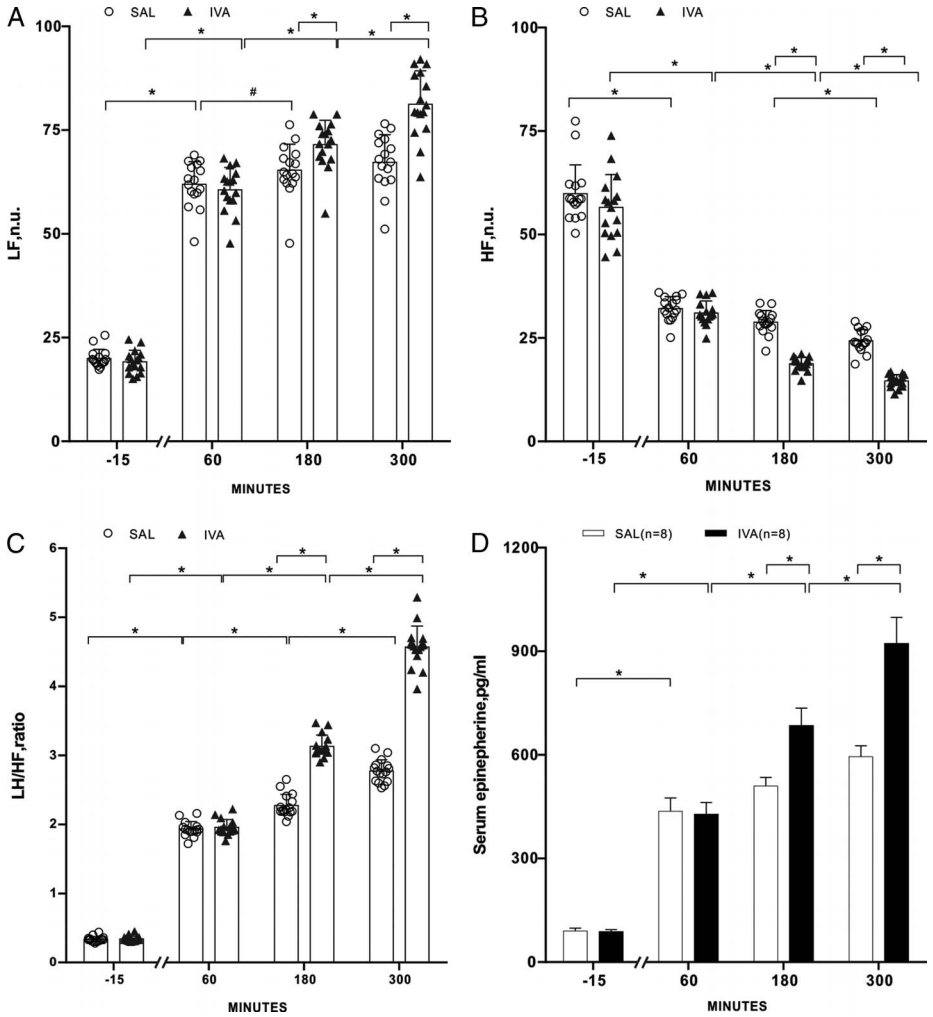

FIG. 4. **Scatter bar and bar graph show the effect of induced HRR on HRV (A–C) and serum epinephrine (D), respectively**. #$P < 0.05$, *$P < 0.01$ versus IVA group. –15, baseline; HF, high frequency; IVA, ivabradine; LF, low frequency; LF/HF, low frequency/high frequency ratio; n.u., normalized unit; SAL, saline placebo.

In the present study, IVA-induced HRR was associated with elevated MAP and SVR. Because IVA itself is devoid of vasoactive effect, stronger vasoconstriction of resistance arteries due to increased sympathetic activity and epinephrine production was the only explanation. Microcirculatory derangement as expressed by poorer PVD and MFI could also be attributed to a similar mechanism. Excessive epinephrine surely aggravates myocardial ischemia and dysfunction with β1 and α-adrenergic actions and leads to increased severity of myocardial injury as indicated by elevated serum cTnI concentrations and a strikingly shortened survival duration as shown in the present study, a process that has been supported by earlier experiments in animals (3,21).

With the "pure bradycardic agent" effect of IVA (6,22) and no disturbance from exogenous epinephrine, the present bench study in essence uncovered the direct links between stunned myocardium, PR HR, and PR myocardial function. Those results, being the opposite of our initial hypothesis and in stark contrast to those proven beneficial effects of IVA aforementioned, demonstrate that the long-duration rapid HR PR, which impartially corresponds to the stunned myocardium, is essential for preserving PR myocardial function. This inference is completely in accord with heart failure physiology and also supported by the earlier finding that more severe stunning of the myocardium due to longer duration of ventricular fibrillation results in faster HR PR (3). Therefore, it is the stunned myocardium not the HR PR that should be the therapeutic target for post-CA. Spontaneous reduction in such HR may be an indicator of an improved stunned myocardium, whereas artificial reduction ahead of the improvement in myocardial stunning as a breach of the physiological law (23) certainly leads to worse PR outcomes.

Correspondingly, although the HR PR is clinically affected by HR-reducing factors and HR-accelerating ones, a synthetic rapid HR close to the original level should be preserved until reversal of myocardial stunning (24). However, in the real world, HR PR has not had much attention attached to it. Most notably, bradycardia unintentionally induced by bedside interventions for treating CA is very common. Their negative effect *via* HRR on PR myocardial function is likely to counteract their proven benefits and then result in compromised outcomes. Active HR compensation therefore is of great necessity once such situation occurs.

Lidocaine and amiodarone are widespread guideline-supported antiarrhythmics for shock-refractory ventricular fibrillation/pulseless ventricular tachycardia CA (25) for their efficacies in improving ROSC rates and/or the survival to hospital admission. Despite these beneficial short-term outcomes, this fails to achieve a better long-term prognosis. The present finding sheds light for the first

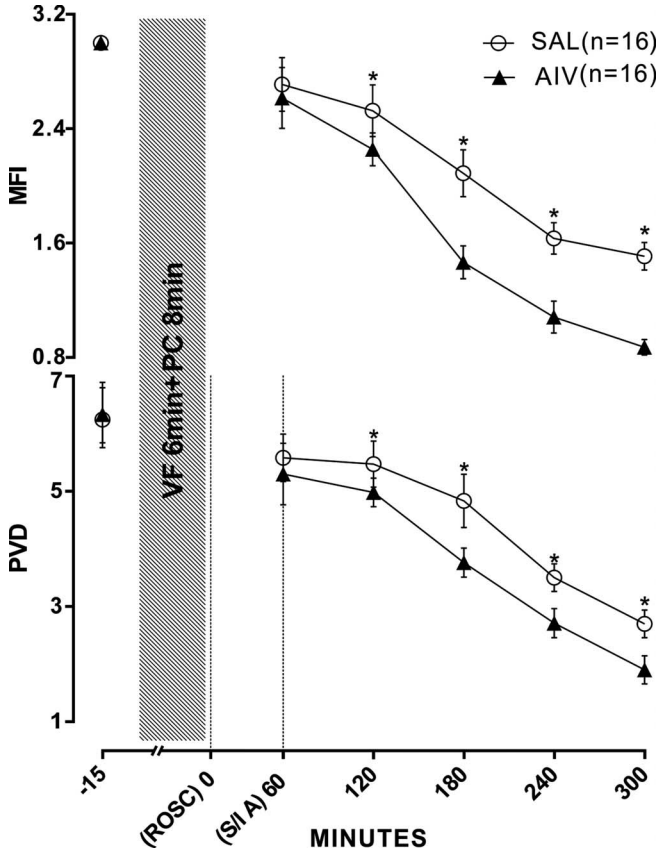

FIG. 5. **Scatterplots show the effect of ivabradine-induced HRR on microcirculation**. *P < 0.01 versus IVA group. –15, baseline; IVA, ivabradine; MFI, microvascular flow index; n = n minutes postresuscitation; PVD, perfused vessel density; ROSC, return of spontaneous circulation; SAL, saline placebo.

time on this previously confusing discrepancy. One particular concern is that in the well-known "Amiodarone, Lidocaine, or Placebo in Out-of-Hospital Cardiac Arrest" study (26), it shows that although both amiodarone and lidocaine exhibit a significant HRR effect, it is greater with amiodarone, resulting in "a greater need for temporary cardiac pacing after receipt of amiodarone (4.9%) than after receipt of lidocaine (3.2%) or placebo (2.7%)." In an earlier swine model, amiodarone administered during CPR resulted in worse PR hemodynamics associated with significant HRR (27). On the basis of our finding, both PR bradycardia (slow HR) unintentionally induced by accumulated large doses of amiodarone/lidocaine repeatedly administered during CPR and the preferred frequencies of approximately 60 bpm of transvenous temporary cardiac pacing for treating this situation are far from the needed level, resulting in marked decrease in CO, effectively offsetting their efficacy in restoring electrical stability. The class IIb (weak) strength of recommendation for lidocaine or amiodarone administration during CPR in the guideline (25) is therefore justified. For those survivors with bradycardia PR, a timely HR compensation presumably reverses the situation and improves long-term outcomes. Transvenous temporary cardiac pacing is held to be the preferred approach because of its pure efficacy in reaching targeted frequencies and controlled durations despite the fact that atropine or β-adrenergic agonists with HR-accelerating effects are also conditionally recommended (28).

Targeted temperature management (TTM) is another guideline-supported therapy for treating comatose and pediatric CA survivors that helps to achieve neurologically favorable survival. Although therapeutic hypothermia is of benefit for myocardial protection (29), with a slow HR being a physical response to low body temperature, TTM at low temperatures may contribute to severe bradycardia followed by decreased CO and intense vasoconstriction, which could negate its neuroprotective effect and so compromise the overall outcome. In fact, earlier clinical observations have revealed the negative effect of hypothermia-induced HRR on PR myocardial function where CO is decreased by 6% to 7% following HRR for each 1°C decrease in core temperature (30) and by 20% to 25% following TTM at 33°C mainly due to a 15% to 20% reduction in HR (31). Therefore, despite the earlier judgment that bradycardia during TTM may indicate lower mortality and favorable neurologic outcomes (32), it does not mean that the improved outcomes are attributed to a slow HR. The present finding favors TTM at 36°C of brief duration over TTM at lower temperatures with prolonged duration to avoid severe bradycardia. This is supported by another earlier finding in a rat model of CPR showing that TTM at 33°C for 2 h achieves better PR myocardial and cerebral function with unchanged HR than TTM at 33°C for 5/8 h with

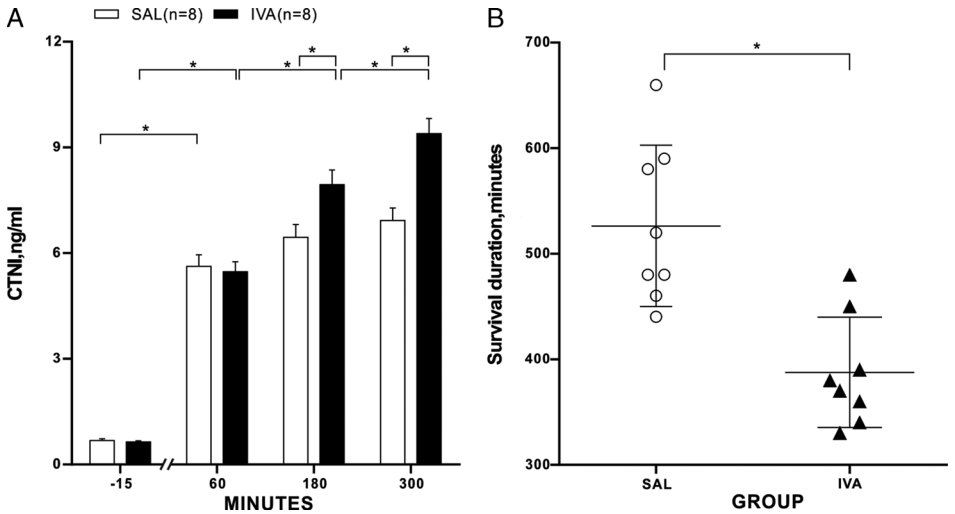

FIG. 6. **Bar graph and scatterplots show the effect of induced HRR on serum cTnI (A) and survival duration (B), respectively**. *P < 0.01 versus IVA group. –15, baseline; cTnI, cardiac troponin I; IVA, ivabradine; SAL, saline placebo.

significantly lower HR (33). It may also help to explain two clinical findings that TTM at 33°C does not benefit out-of-hospital CA survivors more than TTM at 36°C (34), and TTM at 33°C is not superior to targeted normothermia in improving long-term mortality (35). Heart rate compensation using transvenous temporary cardiac pacing aforementioned during TTM at low temperatures presumably also achieves preserved CO and neurological benefit and therefore improves long-term prognosis.

LIMITATIONS

There were certain limitations to our study. First, although the unmodified rapid HR PR exists in principle, in practice there are many HR-modifying interventions used, which means that it is not usually possible to observe this phenomenon unaffected by other agents or treatments.

- The HR of rat is very susceptible to depth of anesthesia, blood temperature, and many interventions with HR-affecting effects. It is not surprising at all that HR after ROSC is equal to or even lower than the baseline level in some investigations on rats. With light narcosis and blood temperature at 36.5°C to 37.5°C, the sustained rapid HR PR originally occurred in our earlier investigation (3) and the present study.
- As far as the porcine model of epinephrine-assisted CPR is concerned, the HR after ROSC is inevitably disturbed by exogenous epinephrine (14).
- For out-of-hospital CA patients, HR after ROSC actually varies with baseline level and the numerous factors, so the unmodified rapid HR PR is always obscured.

Those are why the condition being studied is not one likely to be studied in humans or porcine model. Second, because detailed HR and hemodynamics data are not included or listed in those clinical trials (26,34,35), further analyses on the relationship based on HR change between antiarrhythmics, therapeutic hypothermia, and PR outcomes are hardly achieved. Third, on the basis of preliminary test results, only one single group of IVA 0.5 mg/kg with an HRR ($\Delta \approx -30$ bpm) was set for comparative observation. Finally, for technical reasons, investigations of stretch-tension relation and myofibrillar calcium sensitivity in myocardium PR were not included.

CONCLUSION AND PERSPECTIVE

The present study using a rat model of CPR showed that IVA-induced HRR increases the severity of PR myocardial dysfunction and shortens survival duration, demonstrating that the sustained rapid HR PR is essential for preserving postresuscitation myocardial function.

In current clinical practice, bradycardia PR due to HRR induced by amiodarone/lidocaine or therapeutic hypothermia for treating out-of-hospital CA is very common. It could cause worsened PR myocardial function and thus counteract their efficacies leading to compromised long-term outcomes. However, there is yet no any guideline or expert consensus giving definitive guidance on the appropriate HR and its duration after restoration of spontaneous circulation for out-of-hospital CA survivors despite the fact that guidelines have recommendations on how to manage "symptomatic" bradycardia. Intensive caregivers should pay enough attention to HR and provide appropriately rapid frequency even if "asymptomatic" bradycardia PR occurs.

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