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The Individual Role of Sarcolemmal and Mitochondrial K_{ATP} Channels Opening During Cardiopulmonary Resuscitation in a Porcine Model Treated with Levosimendan

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Abstract

Objective: We investigated the individual role of the sarcolemmal K_{ATP} ($sarcK_{ATP}$) channel and the mitochondrial K_{ATP} ($mitoK_{ATP}$) channel opening during cardiac arrest and resuscitation by levosimendan administration to improve post-resuscitation myocardial function in a porcine model.

Materials & Methods: Twenty pigs were randomized into 4 groups: 1) levosimendan (LEVO) + HMR-1098 ($sarcK_{ATP}$ channel blocker): HMR-1098 3 mg/Kg was injected 30 min before inducing ventricular fibrillation (VF) and levosimendan 40 μg/kg was injected 3 min after inducing VF (VF 3); 2) LEVO + 5-HD ($mitoK_{ATP}$ channel blocker): 5-HD 5 mg/Kg was injected 30 min before inducing VF and levosimendan 40 μg/kg was injected at VF 3; 3) LEVO: levosimendan 40 μg/kg was injected at VF 3; 4) control: an equal volume of saline placebo was injected at VF 3. VF was induced by intraluminal balloon occlusion of the left anterior descending coronary artery. After 7 min of untreated VF, CPR was initiated for 5 min followed by defibrillation. Resuscitated animals were observed for 4 hrs. Myocardial function was assessed by echocardiographic doppler measurements. To examine myocardial protection, we assessed measurements obtained from a PC-based data acquisition system, supported by CODAS/WINDAQ hardware/software, a stat profile analyzer and a lactic acid analyzer.

Results: Pre-treatment of the $sarcK_{ATP}$ channel blocker increased ventricular arrhythmia and the number of defibrillation shocks required. Blocking the $mitoK_{ATP}$ channel completely abolished myocardial protective effects of LEVO. Beneficial effects of decreased ST segment elevation, reduced production of myocardial H+, and lactate and CO_2 as observed following administration of LEVO no longer existed after blocking either channel.

Conclusion: Activation of both channels by LEVO provides myocardial protective mechanisms. Activation of the $sarcK_{ATP}$ channel reduces post-resuscitation ventricular arrhythmia, while activation of the $mitoK_{ATP}$ channel improves myocardial mechanical function. Reduced myocardial H+, lactate and CO_2 production was observed after activation of both channels.

Keywords: Post-resuscitation; Cardioprotection; Sarcolemmal K_{ATP} channel; Mitochondrial K_{ATP} channel

Abbreviations: K_{ATP}: ATP-sensitive potassium channels; CPR: Cardiopulmonary resuscitation; IPC: Ischemic pre-conditioning; LEVO: Levosimendan; $sarcK_{ATP}$: Sarcolemma ATP-sensitive potassium channels; $mitoK_{ATP}$: Mitochondrial ATP-sensitive potassium channels; FiO_2: Inspired O_2 fraction; $P_{ET}CO_2$: End-tidal PCO_2; LAD: Left anterior descending; VF: Ventricular fibrillation; HMR-1098: $sarcK_{ATP}$: Channel blocker; 5-HD: 5-hydroxydecanoate; $mitoK_{ATP}$: Channel blocker; PC: Precordial compression; CPP: Coronary perfusion pressure; EF: Ejection fraction; FAC: Fractional area change; CO: Cardiac output; PC 3: 3 min of PC; PR 5: Post-ressuscitation 5 min; PVB: Premature ventricular beat; SD: Standard deviation; SEM: Standard error of the mean; ROSC: Return of spontaneous circulation

Introduction

Although 40% of victims of cardiac arrest are initially resuscitated successfully, only 5-10% of those victims survive [1-4]. The majority of those initially resuscitated died within the first 72 hrs due to post-resuscitation myocardial dysfunction or anoxic encephalopathy. In previous studies, we demonstrated that pharmacological opening of ATP-sensitive potassium (K_{ATP}) channels significantly reduced post cardiopulmonary resuscitation (CPR) myocardial injury and improved post-resuscitation survival, which mimics the myocardial protective effects of ischemic pre-conditioning (IPC) [5]. We also demonstrated that levosimendan (LEVO), as a non-specific K_{ATP} channel opener and calcium sensitizer, attenuated myocardial ischemic injury, minimized ventricular ectopy and enhanced post-resuscitation myocardial contractility in porcine and rat models of cardiac arrest and resuscitation [6-11]. These cardioprotective effects, however, were completely abolished by a non-selective K_{ATP} channel blocker, glibenclamide [12].

There are two K_{ATP} channel subtypes in the myocardium; one is located in the sarcolemma ($sarcK_{ATP}$) and the other in the inner membrane of the mitochondria ($mitoK_{ATP}$) [13]. Our early research did not provide a mechanism of these two K_{ATP} channels in post-resuscitation myocardial protection. The purpose of this study was to determine the specific role of $sarcK_{ATP}$ or $mitoK_{ATP}$ channel modulation on post-resuscitation myocardial function in a porcine model of ischemic cardiac arrest and resuscitation. We hypothesized that the mechanisms of myocardial protection are different following the activation of the two channels; the $sarcK_{ATP}$ channel activation would have more of an effect on reducing post-resuscitation ventricular arrhythmias, while the $mitoK_{ATP}$ channel activation would reduce myocardial metabolism and improve post-resuscitation myocardial mechanical function.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee of the Weil Institute of Emergency and Critical Care Research. Humane care was rendered to all animals in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health [14,15].

Animal preparation

Twenty male domestic pigs weighing 35 - 40 kg were fasted overnight except for free access to water. Anesthesia was initiated by intramuscular injection of ketamine (20 mg/kg) and completed by ear vein injection of sodium pentobarbital (30 mg/kg). Additional doses of sodium pentobarbital (8 mg/kg) were injected to maintain anesthesia.

A cuffed endotracheal tube was advanced into the trachea. The animals were mechanically ventilated with a volume controlled ventilator (Model MA-1, Puritan-Bennett, Carlsbad, CA) and with a tidal volume of 15 ml/Kg, peak flow of 40 L/min, and an inspired O_2 fraction (FiO2) of 0.21. End-tidal PCO_2 ($P_{ET}CO_2$) was monitored with an infrared analyzer (Model 01R-7101A, Nihon Kohden Corp, Tokyo, Japan). Respiratory frequency was adjusted to maintain $P_{ET}CO_2$ between 35 and 40 mm Hg.

For the measurement of left ventricular function, a 5.5/7.5 Hz biplane with Doppler transesophageal echocardiographic transducer with 4-way flexure (Model 21363A, Hewlett-Packard Co., Medical Products Group, Andover, MA) was advanced from the incisor teeth into the esophagus for a distance of approximately 35 cm. For the measurement of aortic pressure, a fluid filled catheter was advanced from the left femoral artery into the thoracic aorta. For the measurements of right atrial pressure, pulmonary arterial pressure and blood temperature, a 7-French pentalumen thermodilution-tip catheter was advanced from the left femoral vein and into the pulmonary artery. A 7-French catheter was advanced from the left cephalic vein into the great cardiac vein for measurements of great cardiac vein blood gases and lactate.

Myocardial ischemia was induced in a closed-chest preparation by intraluminal occlusion of the left anterior descending (LAD) coronary artery between the first and second diagonal branches. The right common carotid artery was isolated and a 7-French Swan-Ganz catheter was inserted. The LAD coronary artery occlusion was guided under contrast guidance. The catheter was inserted into the left main coronary artery and was rotated and pushed into position at the proximal end, just beyond the first diagonal branch of the LAD coronary artery. The balloon was inflated with air to occlude the vessel completely, as verified with angiography. 2,500 IU of heparin was injected into the LAD coronary artery as soon as the artery was occluded. The catheter was not removed until the start of resuscitation.

Experimental procedures

Forty-minutes prior to ventricular fibrillation (VF), animals were randomized by the *Sealed Envelope Method*. Investigators were blinded to randomization. The sarcK_{ATP} channel blocker (HMR-1098) (3 mg/Kg) (Aventis, Frankfurt, Germany) or the mitoK_{ATP} channel blocker 5-hydroxydecanoate (5-HD, 5 mg/Kg) (Sigma, US) was injected as a bolus into the right atrium 30 min prior to start of VF. Cardiac arrest was induced by LAD coronary artery occlusion. The duration between LAD coronary artery occlusion and VF was 5.57 ± 1.99 min. Mechanical ventilation was discontinued after the onset of VF. At the end of 7 minutes of untreated VF, the LAD coronary artery occlusion was released and precordial compression (PC) was initiated with a pneumatic piston-driven chest compressor (Thumper, Model 1000, Michigan Instruments, Grand Rapids, MI). Coincident with the start of PC, animals were mechanically ventilated with a tidal volume of 15 ml/kg and FiO_2 of 1.0. Precordial compression was programmed to provide 100 compressions-per-minute and synchronized to provide a compression/ventilation ratio of 5:1 with equal compression-relaxation intervals, i.e., a 50% duty cycle. The compression force was adjusted to decrease the anterior-posterior diameter of the chest by 25%. After 5 min of precordial compression, defibrillation was attempted with a 150 J biphasic waveform shock delivered between the right infraclavicular area and the cardiac apex. If an organized cardiac rhythm with mean aortic pressure of more than 60 mmHg persisted for an interval of 5 min or more, the animal was regarded as successfully resuscitated.

Animals were randomized into the following groups: 1) HMR-1098 3 mg/Kg bolus 30 min prior to starting VF and levosimendan 40 μg/kg bolus 3 min after induced VF 3); 2) 5-HD 5 mg/kg bolus 30 min prior to starting VF and levosimendan 40 μg/kg bolus at VF 3; 3) a bolus dose of levosimendan 40 μg/kg) was injected at VF 3; 4) an equal volume of saline placebo.

Measurements were continued for 4 hrs after successful resuscitation. The experimental procedures are summarized in Figure 1. At the end of 4 hrs, animals were euthanized by intravenous injection of 150 mg kg-1 pentobarbital. A necropsy was performed to document injuries to the bony thorax and the thoracic or abdominal viscera.

Measurements

Dynamic data, including aortic, right atrial, pulmonary arterial pressures and $P_{ET}CO_2$, together with electrocardiogram were continuously measured and recorded on a PC-based data acquisition system, supported by CODAS/WINDAQ hardware/software as previously described [16]. During CPR, coronary perfusion pressure (CPP) was digitally computed as the difference between aortic and right atrial diastolic pressures.

Echocardiographic measurements were obtained with the aid of a Hewlett-Packard Sonos 2500 echocardiographic system utilizing a 5.5/7.5 Hz biplane Doppler transesophageal echocardiographic transducer with 4-way flexure (Model 21363A, Hewlett-Packard Co., Medical Products Group, Andover, MA). For the long axis, a 2 or 4 chamber view was obtained. Left ventricular end-systolic and diastolic volumes were calculated by discs (Acoustic Quantification technology, Hewlett-Packard, Andover, MA). From these, ejection fraction (EF), fractional area change (FAC) and cardiac output (CO) were computed. These measurements served as a quantitative indicator of myocardial

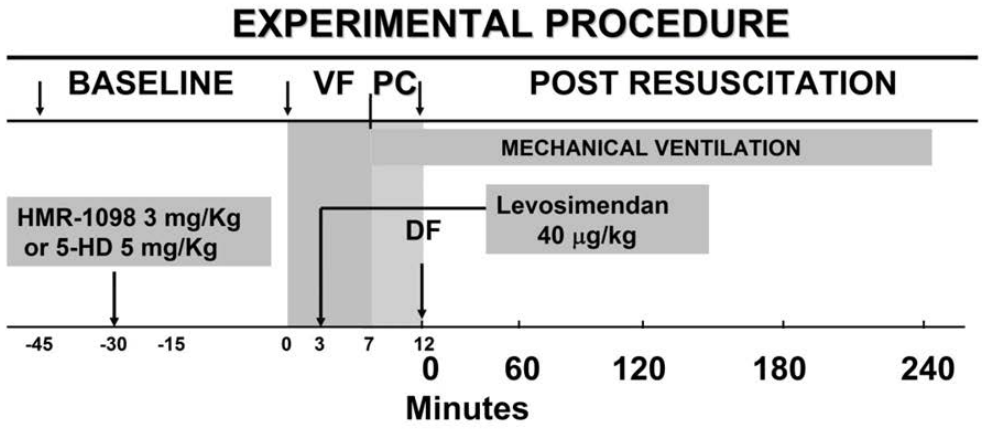

Figure 1: Experimental procedure. Time relations of baseline measurements (-45 min), inducing VF (0 min), injection of HMR-1098 or 5-HD (-30min), injection of levosimendan or vehicle three min after onset of VF(VF 3), and CPR, including DF at 12 min. The duration of mechanical ventilation and timing of intermittent measurements are also shown.

VF: Ventricular fibrillation; CPR: Cardiopulmonary resuscitation; DF: Defibrillation.

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function. Then, post- resuscitation myocardial injury manifested as decreased CO, EF and FAC, were observed after return of spontaneous circulation (ROSC) in all animals and compared to baseline and between groups. Aortic, mixed venous and great cardiac venous blood gases, hemoglobin and oxyhemoglobin were measured from 200 µl aliquots of blood with a stat profile analyzer (ULTRA C, Nova Biomedical Corporation, Waltham, MA) adapted for porcine blood. Arterial and great cardiac venous blood lactate was measured with a lactic acid analyzer (Model 23L, Yellow Springs Instruments, Yellow Springs, OH). These measurements were obtained 45 min prior to cardiac arrest, at 3 min of PC (PC 3) and at hourly intervals after resuscitation for a total of 4 hrs and were analyzed and compared from each group. The elevated ST segment at post-resuscitation 5 min (PR 5) and the premature ventricular beat (PVB) during the first 10 min after resuscitation were measured and analyzed. Differences of pH, lactate and CO_2 between great cardiac vein and aorta at PC 3 were also analyzed.

Statistical analyses

All data were presented as mean ± standard deviation (SD) or standard error of the mean (SEM). Differences of hemodynamic and metabolic measurements among groups were analyzed by ANOVA using the Scheffe method for multiple comparisons. Differences within groups were analyzed with ANOVA measurement. A value of $p < 0.05$ or $p < 0.01$ was regarded as significant.

Results

A total of 20 animals were utilized and reported. Baseline hemodynamics, $P_{ET}CO_2$, blood analytical measurements, myocardial function and the duration from LAD coronary artery occlusion to VF did not differ among groups (Table 1). There were no differences in CPP and $P_{ET}CO_2$ during CPR among groups (Table 2).

All animals were resuscitated after CPR. However, a significantly greater number of shocks were required for restoring spontaneous circulation in animals treated with LEVO + HMR-1098 when compared with LEVO, LEVO+5-HD or placebo treated animals ($p = 0.0138$, 0.0177, and 0.0284, respectively) (Table 2, Figure 2A). The incidence of PVBs during the first 5 min of post-resuscitation was significantly reduced in the LEVO and LEVO+5-HD treated groups compared with the LEVO+ HMR-1098 group (p = 0.0015 and 0.0038, respectively) and controls (p = 0.0181) (Table 2, Figure 2B). The magnitude of ST segment elevation at PR 5 was significantly greater in both the LEVO+ HMR-1098 group and the LEVO+5-HD group when compared to either the LEVO group ($p = 0.0035$ and 0.0003, respectively) or controls (p =0.0130 and 0.0044, respectively) (Table 2, Figure 2C).

Significantly less pH, PCO_2 and lactate between aortic and great cardiac vein blood were observed in the LEVO group when compared to the LEVO+HMR 1098 group ($p = 0.0252$,0.0012, and 0.0064, respectively), the LEVO+5-HD group ($p = 0.0098$,0.0133 and 0.0247, respectively) and controls ($p = 0.0008$, 0.0002 and 0.0170, respectively) (Table 1, Figure 2D-2F).

As previously reported, post-resuscitation myocardial dysfunction, including decreased CO, EF and FAC, were observed and analyzed after return of spontaneous circulation (ROSC) in all animals when compared to baseline [9-12]. However, the severity of post-resuscitation myocardial dysfunction was significantly lower in LEVO treated animals when compared with controls. The beneficial effects of LEVO, however, were completely abolished by pre-treatment with 5-HD but not HMR-1098. The EF and FAC of LEVO and LEVO+HMR-1098 treated animals returned to 92.72 ± 3.85, 89.40 ± 13.62% and 82.91 ± 8.99%, 97.37 ± 30.03% of baseline at 4 hrs after ROSC; whereas the EF, FAC in LEVO+5-HD treated and controls was only 61.50 ± 11.92%, 57.07 ± 6.42% ($p = 0.0003$, 0.0017 *vs.* LEVO, $p = 0.0069$, 0.0191 *vs.* LEVO+HMR-1098, respectively) and 62.58 ± 5.37%, 54.10 ± 18.67% ($p = 0.0000$, 0.0052 *vs.* LEVO, $p = 0.0020$, 0.0152 *vs.* LEVO+ HMR-1098, respectively). CO of LEVO and LEVO+HMR-1098 treated animals returned to 100.14 ± 16.83 % and 89.70 ± 14.24% of baseline at 4 hrs after ROSC, which had no significant difference, whereas CO in LEVO+5-HD treated and controls was only 75.21 ± 16.05% and 73.71 ± 18.42 % (p=0.0217 and 0.0228 *vs.* LEVO, respectively) (Figure 3).

No gross abnormalities and injuries related to CPR were identified during necropsy.

Table 1: Measurements and parameters during baseline.

	Time	LEVO	LEVO+HMR	LEVO+5-HD	Control
HR,beats.min-1	BL	142 ± 9.3	144 ± 30.3	140.8 ± 36.9	135.60 ± 17.8
MAP, mmHg	BL	126.6 ± 16.7	118.8 ± 11.0	128.2 ± 31.7	118.0 ± 13.1
PETCO2,mmHg	BL	37.6 ± 2.2	38.3 ± 1.6	39.1 ± 2.4	37.7 ± 1.8
EF,%	BL	63.8 ± 2.4	66.2 ± 2.2	64.6 ± 3.8	63.8 ± 2.2
CO, L/min	BL	5.34 ± 0.31	5.72 ± 0.60	5.23 ± 0.38	5.40 ± 0.60

HR: Heart rate; MAP: Mean arterial pressure; PETCO2: End-tidal PCO2; EF: Ejection fraction; CO: Cardiac output.

Table 2: Hemodynamic and biochemistry data during VF and resuscitation.

	Time	LEVO	LEVO+HMR	LEVO+5-HD	Control
LAD occlusion to VF, min, s	LAD occlusion to VF	5min 36s ± 2min 59s	6min 32s ± 2min 28s	6min 27s ± 2min 20s	6min 33s ± 2min 43s
CPP,mmHg	PC3	21.6 ± 2.3	20.8 ± 4.5	23.4 ± 3.3	23.6 ± 2.1
PETCO2,mmHg	PC3	21.8 ± 6.3	19.5 ± 2.7	21.1 ± 2.7	17.1 ± 5.9
PH (A-C) value	PC3	0.52 ± 0.06ΔΔ	0.68 ± 0.15*	0.72 ± 0.15**	0.74 ± 0.09**
Lactate (C-A), mmol/l	PC3	0.92 ± 0.93	3.30 ± 1.39**	2.48 ± 1.17*	2.46 ± 0.98*
CO2, mmHg	PC3	52.7 ± 12.1ΔΔ	99.1 ± 20.4**	88.9 ± 24.6*	104.4 ± 15.4**
Shock numbers	CPR	4.8 ± 3.4 #	17.2 ± 9.7Δ	4.0 ± 3.9#	5.8 ± 3.6 #
PVBs	PR0 to PR5	9.4 ± 4.2 ##Δ	24.8 ± 6.5 **Δ	11.6 ± 2.3 ##Δ	15.6 ± 4.8 *#
ST segment elevation, mv	PR5	0.10 ± 0.04 ##	0.26 ± 0.08**Δ	0.27 ± 0.05**ΔΔ	0.13 ± 0.07#

LAD: Left anterior descending; min: Minute; s: Second; VF: Ventricular fibrillation; CPP: Coronary Perfusion Pressure; $PETCO_2$: End-tidal PCO_2; A-C: Artery and cardiac vein; C-A: Cardiac vein and artery; PVBs: Premature ventricular beats; PR: Postresuscitation; PC: precordial compression

*p<0.05 vs. LEVO group, **p<0.01 vs. LEVO group; # p<0.05 vs. LEVO +HMR group, ## p<0.01 vs. LEVO+HMR group; Δp<0.05 vs. Control group, ΔΔ p<0.01 vs. Control group; Data expressed as mean ± SD, based on ANOVA test as appropriate.

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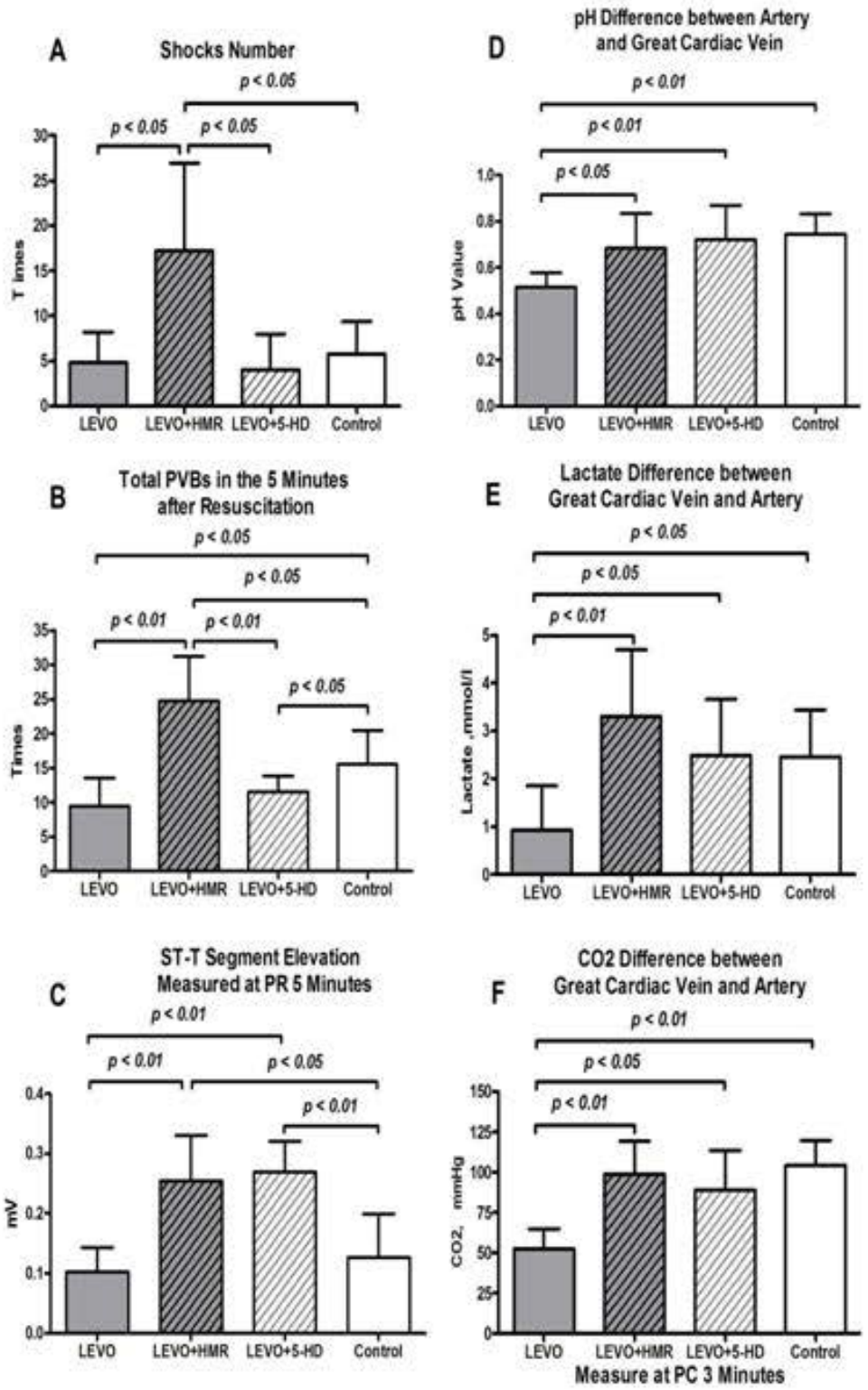

Figure 2: Number of defibrillating shocks, PVBs, and ST elevation among groups (A-C). Value of pH, lactate, and CO_2 differences between great cardiac vein and artery at PC3 among groups (D-F). The PVBs were counted during post-resuscitation 0 to five min. The ST was measured at post-resuscitation five min (PR5).

LEVO: Levosimendan group; LEVO+HMR: Levosimendan+HMR-1098 group; LEVO+5-HD: Levosimendan+5-HD group; Control: Control group (n=5 in each group); PVBs: Premature ventricular beats; PR: Post-resuscitation; PC: Precordial compression; LEVO: Levosimendan. The bar length represents standard deviation (SD).

Discussion

The present study demonstrates different myocardial protective mechanisms following activation of sarcKATP or mitoKATP in a porcine model of CPR. Activation of the sarcKATP channel reduces post-resuscitation ventricular arrhythmia, while activation of the mitoKATP channel improves myocardial mechanical function. However, both channels are involved in reducing myocardial metabolic rate during ischemia. This is a follow-up study from a previous publication, which demonstrated that glibenclamide, a non-selective

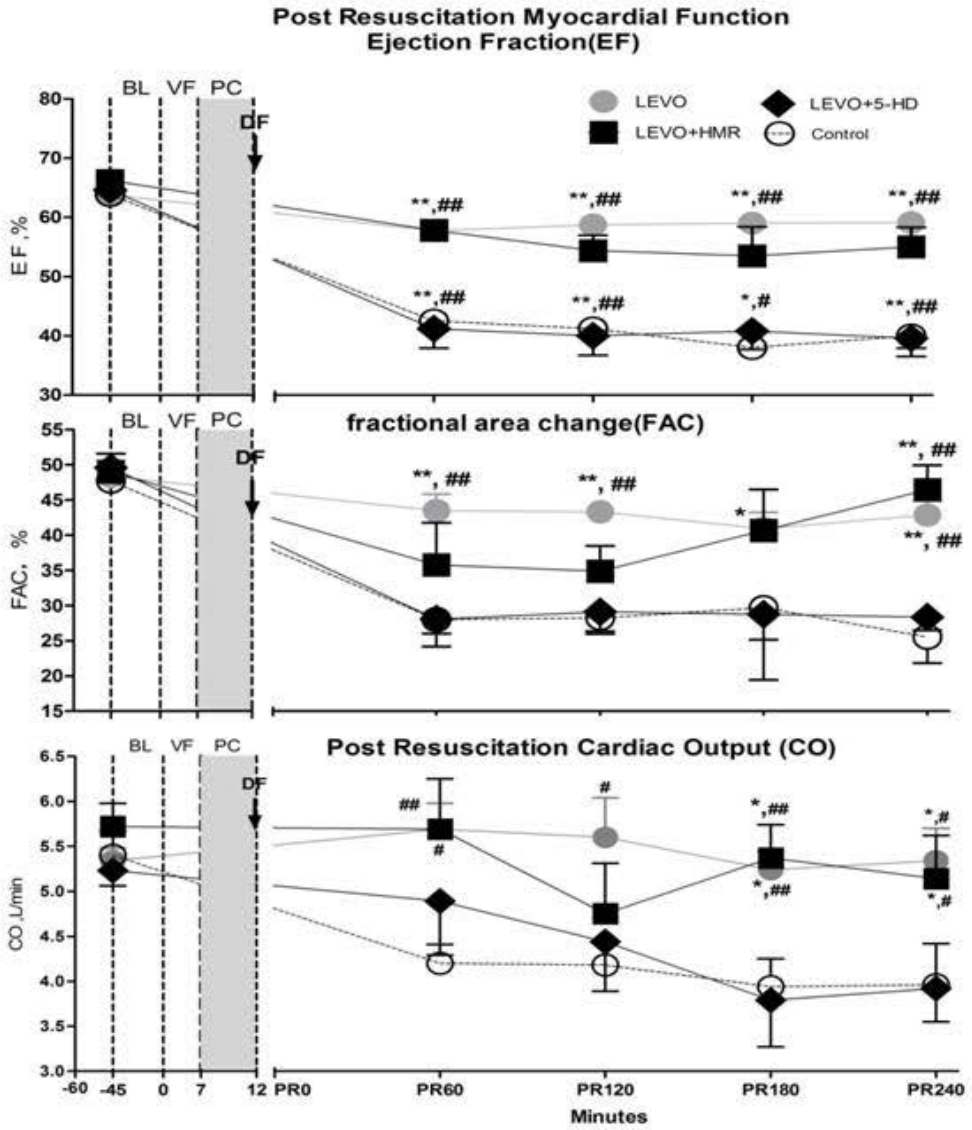

Figure 3: Effect of four interventions on post- resuscitation left ventricular ejection fraction (EF), fractional area change (FAC), and Cardiac output (CO) values represent mean values and the bars SEM.

BL: Baseline; DF: Defibrillation; PC: Precordial compression; VF: Ventricular fibrillation.

LEVO in the solid grey circle: Levosimendan group; LEVO+HMR in the solid black square: Levosimendan+HMR-1098 group; LEVO+5-HD in the solid black diamond: Levosimendan+5-HD group; Control in the clear circle: Control group (n=5 in each group).

**P*<0.05, ***P*<0.01 other groups *vs* LEVO+5-HD group; #*P*<0.05, ##*P*<0.01 other groups *vs* control group.

The bar length represents the standard error of mean (SEM).

KATP channel blocker, completely abolished the cardiac protective effect of levosimendan in a rat cardiac arrest model [9-12].

In the present study, LEVO+ HMR-1098 treated animals required a significantly greater number of defibrillation shocks to restore spontaneous circulation. Those animals also exhibited a greater number of PVBs following resuscitation when compared with the other groups. These results indicate that blocking the sarcK_{ATP} channel during ischemia increases defibrillation threshold and the incidence of reperfusion-induced ventricular arrhythmias. The mechanisms of ischemia-reperfusion induced arrhythmias may be caused by a delay after depolarization resulting from altered Ca^{2+} homeostasis [17]. Opening of the sarcK_{ATP} channel by LEVO during ischemia and reperfusion increases the efflux of potassium from myocytes during repolarization. Increases in extracellular potassium reduce the duration of action potential and the time available for voltage-dependent calcium influx. Less cytosolic calcium would account for lower defibrillation thresholds and less incidence of ventricular arrhythmias as observed in animals treated with LEVO alone [13,18-22]. This phenomenon also suggests that we could measure the energy required to terminate VF and then determine whether this energy differed among groups.

In the present study, significantly greater ST segment elevation was observed immediately following resuscitation in animals treated with LEVO+HMR 1098 or LEVO+5-HD when compared with LEVO alone or control. Elevation of the ST is hallmark of acute transmural myocardial ischemia reflecting dispersion of repolarization [23]. It is a typical electrocardiogram phenomenon in our experimental cardiac arrest and resuscitation models. Global myocardial ischemia leads to intracellular and extracellular acidosis, extracellular K^+ accumulation, intracellular ATP depletion, overload of intracellular Ca^{2+}, reduced excitability at rest potentials, delayed recovery of excitability and shortened transmembrane action potential. These events lead to injury currents generated between ischemic and non-ischemic myocytes, shifts

in electrical resistance from cell-to-cell and electrical inhomogeneity within the heart, leading to the shifting of ST segments in the ECG [21,23-26]. The sarcK_{ATP} channel blocker HMR - 1098 prevents shortening of the duration of the action potential and abolishes optimization of cardiac energy consumption induced by the sarcK_{ATP} channel opener LEVO. This could increase extracellular K^+ and intracellular Ca^{2+}, thereby increasing myocardial ischemic injury [8,13,18,26,27]. Opening the mitoK_{ATP} channel resulted in activation of the respiratory chain and an improvement in cellular bioenergetics. When mitoK_{ATP} channel blocker 5 - HD was administered, mitochondrial metabolism was blocked and resulted in intracellular acidosis [6,17,26,27]. Accordingly, both 5-HD and HMR-1098, through different mechanisms, increased the severity of myocardial ischemic injury as reflected in an elevated ST segment.

This study demonstrated that the non-selective K_{ATP} channel opener, LEVO, significantly reduced myocardial metabolic rate when administered during ischemia; this resulted in significantly reduced pH, CO_2 and lactate. When blocking either sarcK_{ATP} or mitoK_{ATP} channels, the effects of reduced cardiac end products including H+, lactate and CO_2 following levosimendan were abolished. Although the relationship between sarcK_{ATP} channel opening and myocardial metabolism during ischemia remains to be investigated, increases in metabolic end products of the heart due to 5-HD may directly result from its specific mitoK_{ATP} channel blocking action, which interrupts mitochondrial function, the respiratory chain and cellular bioenergetics [26-30].

This may explain poor post-resuscitation myocardial function in animals treated with LEVO + 5-HD. Levosimendan is a myofilament Ca^{2+} sensitizer with inotropic effects and the present study indicates that its inotropic actions are closely related to the mitoK_{ATP} channel. Although animals treated with LEVO + HMR - 1098 demonstrated similar myocardial metabolic conditions as those treated with LEVO + 5-HD, the post-resuscitation myocardial function was significantly improved to the same extent as animals treated with LEVO alone. Our results indicate that only mitoK_{ATP} channel activation is closely related to improvement of myocardial function following administration of LEVO. Other studies have demonstrated that the sarcK_{ATP} channel blocker HMR 1098 has little or no effect on contractility during ischemia [13,18,27,31]. Mitochondria have an intimate role in cell survival by maintaining or enhancing ATP synthesis. The opening of the mitoK_{ATP} channel may partially restore membrane potential, allowing for further extrusion of H^+, forming a more favorable electrochemical gradient for ATP synthesis. When the mitoK_{ATP} channel was blocked, preservation of ATP provided by K_{ATP} channel openers was depleted [6,13,27-29]. This directly resulted in decreased myocardial systolic and diastolic function.

The present study had some limitations: Additional control groups of treatment with either the sarc or mitoK_{ATP} blocker alone were not set; therefore, the effects of co-treatment of levosimendan with either blocker could not be compared to treatment with blocker alone. The decision to not have control groups of treatment with either the sarc or mitoK_{ATP} blocker alone was based on previous publications [10-12,18,19,21], some of which have demonstrated that blocking either sarc or mito K_{ATP} channels may have multiple effects on myocardial injury. Our previous studies demonstrated that levosimendan attenuated myocardial ischemic injury, minimized ventricular ectopy and enhanced post-resuscitation myocardial contractility in both porcine and rat models of cardiac arrest and resuscitation. These cardioprotective effects were completely abolished by a non-selective K_{ATP} channel blocker, glibenclamide. The present study further intended to focus on the individual role of sarcK_{ATP} channel and mitoK_{ATP} channel opening during cardiac arrest and resuscitation by using the sarcK_{ATP} channel blocker and mitoK_{ATP} channel blocker HMR-1098 and 5-HD, respectively. We demonstrated the results by setting the minimum amount of groups necessary to conduct the study [14,15].

Conclusion

We therefore conclude that LEVO significantly reduces myocardial metabolic rate during global ischemia of cardiac arrest and resuscitation and decreases the incidence of post- resuscitation ventricular arrhythmia and severity of myocardial dysfunction. Its effects on reduced incidence of post-resuscitation arrhythmias is due to sarcK_{ATP} channel activation, while its effects on improved myocardial function is due to mitoK_{ATP} channel activation. However, both channels are involved in the reduction of myocardial metabolic rate.

Contributors

Lisa Luna contributed to the editing of this manuscript.

Conflict of Interest

The authors do not have any financial or personal conflicts of interest to disclose. The authors have read the Journal's policy and do not have any conflicts of interest. The authors have no financial or personal relationships with organizations stated in this paper.

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