Esmolol Added in Repeated, Cold, Oxygenated Blood Cardioplegia Improves Myocardial Function After Cardiopulmonary Bypass

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Objective: This study investigated if the β-receptor blocking agent esmolol, added to standard oxygenated blood cardioplegia, improved myocardial function after weaning from bypass.

Design: A block-randomized, blinded study.

Setting: A university laboratory.

Participants: Twenty anesthetized pigs, Norwegian Landrace.

Interventions: After cardiopulmonary bypass, cardiac arrest was induced with cold (12°C), oxygenated blood cardioplegia, enriched with either esmolol or vehicle, repeated every 20 minutes. After 100 minutes the heart was reperfused and weaned.

Measurements and Main Results: Left ventricular function was evaluated with pressure-volume loops, local myocardial function with multilayer strain and strain rate by epicardial short-axis tissue Doppler imaging. One hour after declamping, preload recruitable stroke work did not differ between groups, but increased to 72 ± 3 mmHg in esmolol-treated animals v 57 ± 4 mmHg (p < 0.001) in controls after 3 hours. Radial peak ejection strain rate also was increased by esmolol; 6.0 ± 1.0 s^{-1} v 2.9 ± 0.3 s^{-1} (p < 0.001) in subendocardium and 3.9 ± 0.5 s^{-1} v 2.3 ± 0.2 s^{-1} (p < 0.005) in the midmyocardium. Cardiac index was increased, 4.0 ± 0.2 L/min/m^2 by esmolol v 3.3 ± 0.1 L/min/m^2 for controls (p < 0.05). Isovolumetric relaxation time constant was reduced by esmolol, 23 ± 1 ms v 26 ± 1 ms (p < 0.025). Troponin-T did not differ and was 339 ± 48 ng/L for the esmolol group and 357 ± 55 ng/L for the control group (p = 0.81).

Conclusions: Esmolol added to blood cardioplegia preserved systolic cardiac function during the first 3 hours after reperfusion in a porcine model with 100 minutes of cardioplegic arrest.

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KEY WORDS: esmolol, cardiac function, cardioplegia, cardiopulmonary bypass, beta-blockade

IN NUMEROUS CLINICAL AND EXPERIMENTAL studies, β-adrenergic blocking agents are found to be protective both functionally and structurally in myocardium undergoing ischemia and reperfusion.[1–3] This cardioprotective effect also may be beneficial during cardiopulmonary bypass (CPB) and cardioplegic arrest. However, the cardiodepressive effects of these agents potentially may prolong weaning and lead to an inadequate cardiac response in the postoperative phase. Therefore, special attention has been drawn to the ultra short-acting β-blocker esmolol.

From a cardioprotective point of view, continuous myocardial perfusion with high concentrations of esmolol may be advantageous compared to potassium-based cardioplegia, to facilitate acceptable surgical conditions with little or no myocardial movement.[4–7] Use of lower systemic concentrations of esmolol before, during, or after CPB and cardioplegic arrest in combination with commonly used cardioplegic regimens also have been shown to be beneficial, both experimentally and clinically.[8–10] In isolated hearts both anoxia and ischemia release catecholamine stores, resulting in lipolysis and myocardial tissue damage.[11–13] Crystalloid cardioplegia preserves myocardial catecholamine stores.[14] Esmolol may further reduce the potential ischemic and reperfusion injury by inhibiting the effects of the endogenous release of catecholamines. When added to repeated oxygenated blood cardioplegia, direct intracoronary delivery of esmolol during the cardioplegic perfusions exposes the myocardium to esmolol with a reduced total systemic dose. To the authors' knowledge, only 1 study has used esmolol as an additive to cold blood cardioplegia. In patients urgently operated for unstable angina, the addition of esmolol did not affect myocardial tissue damage judged by troponin-I, CK-MB fraction, and lactate release.[15] However, postoperative cardiac function was not evaluated.

Repeated antegrade, cold, oxygenated, blood cardioplegia is often the preferred method used to facilitate cardioplegic arrest and myocardial protection.[16] In this experimental study, the authors hypothesized that esmolol as an additive to standard potassium-based blood cardioplegia would improve the postoperative cardiac function. Pigs were subjected to CPB and cardioplegic arrest for 100 minutes during tepid general hypothermia in a protocol mimicking a clinical setting. Cardiac function was evaluated with regional and global variables for the first 3 hours after aortic declamping and reperfusion.

METHODS

The experiments were performed using 24 pigs (Norwegian Landrace) of either sex weighing 43 ± 4 kg (SD). Animals were acclimated for at least 7 days. Before surgery animals were fasted overnight, but with access to water. All procedures were performed in accordance with international guidelines described in the European Communities Council Directive of 2010 (63/EU). The experimental protocol was approved by the Norwegian State Commission for Laboratory Animals (project No. 20092088).

After premedication with ketamine, 20 mg/kg, diazepam, 10 mg, and atropine, 1 mg IM, animals briefly were ventilated

with isoflurane, 3% in oxygen, allowing cannulation of 2 ear veins. General anesthesia was induced and maintained by loading doses and continuous infusions of fentanyl, 0.02 mg/kg and 0.02 mg/kg/h, midazolam, 0.3 mg/kg and 0.3 mg/kg/h, vecuronium, 0.4 mg/kg and 0.2 mg/kg/h, and pentobarbital, 15 mg/kg and 4 mg/kg/h. A tracheotomy was performed and the lungs of each pig were ventilated (Julian, Dräger, Lübeck, Germany) with 57% N_2O and oxygen, with a tidal volume of 11 mL/kg. The end-tidal CO_2 was kept within the range of 5.0 to 5.7 kPa (38 to 43 mmHg) by respiratory rate adjustments. Further evaluation of this anesthetic protocol, justifying the safe use of a neuromuscular blocker in young pigs, can be found elsewhere.[17] At the end of the experiments, still under general anesthesia, animals were euthanized by saturated potassium chloride injected into the left atrium.

Surgical Preparation and Instrumentation

The right femoral artery and vein were exposed surgically and cannulated. A suprapubic urine catheter was inserted into the bladder. Following midline sternotomy and pericardiotomy, a band was placed loosely around the inferior caval vein, allowing dynamic preload reductions. A Portex catheter placed into the left atrium was used for microsphere injections. Sutures later needed for bypass cannulation were prepared. A Swan-Ganz catheter (139H-7.5F; Edward Lifesciences Inc., Irvine, CA) was advanced from the right internal thoracic vein to the pulmonary artery, connected to a continuous cardiac output computer (Vigilance I; Edward Lifesciences Inc.) and pressure transducers (SensoNor, Horten, Norway), obtaining central venous and pulmonary artery pressures. Central aortic pressure was measured by a pressure-tip catheter (MPC-500; Millar Corp., Houston, TX) placed in the proximal aorta from the left internal thoracic artery. The pressure-conductance catheter (SPR 788; Millar Corp.), connected to a signal conditioning unit (Sigma 5; CD Leycom, Zoetermeer, the Netherlands), was placed via the apex through the left ventricle and through the aortic valve. Correct position was confirmed by echocardiography (Vivid E9; GE Vingmed Ultrasound, Horten, Norway) and any distal conductance segments with paradox volume signals were excluded. All hemodynamic signals were digitized and recorded with a signal conditioner unit (ACQ-7700; Data Sciences International, St. Paul, MN). Animals were allowed to stabilize for 15 minutes before baseline data were obtained.

Cardiopulmonary Bypass

Animals were cannulated for CPB with an 18F arterial cannula (Medtronic Inc., Minneapolis, MN) in the brachiocephalic artery and a 29F cavoatrial 3-stage cannula (Medtronic Inc.) placed from the right atrial appendage. After tepid CPB flow (90 mL/min/kg) for a short time, mixing blood and prime volume (1200 mL Ringer's acetate), an arterial blood gas was drawn and the aorta was cross-clamped. Oxygenated cold blood cardioplegia, 7% of initial CPB flow (6.3 mL/min/kg), was administered in the aortic root with an initial "high dose" for 3 minutes followed by 2 minutes of "low dose" every 20 minutes (Table 1). Following the first cardioplegia infusion, a 17F left ventricular venting catheter was placed through the left atrium.

Table 1. Final Concentrations in Blood Cardioplegic Perfusate

	High Dose		Low Dose		Total Dose	
K^+	22	mM	14	mM	877	μmol/Kg
Mg^{2+}	16	mM	9	mM	719	μmol/Kg
Cl^-	134	mM	120	mM	2352	μmol/Kg
Procaine	0.8	mM	0.4	mM	37	μmol/Kg
Esmolol	34	μM	19	μM	1.6	μmol/Kg

The animal core temperature was allowed to drift. When reaching 35°C or no later than after 20 minutes, CPB flow was reduced to 72 mL/min/kg. Following the last cardioplegia infusion, CPB flow was reset to 90 mL/min/kg and rewarming was commenced. After 100 minutes the aorta was declamped, and animals weaned from CPB within 20 minutes. If needed, ventricular fibrillations were electroconverted. No other antiarrhythmic intervention was allowed in the protocol. The animals were monitored for 3 hours after aortic declamping.

Design

A block randomized controlled study was performed. Researchers were unaware of the randomization code both during surgery and analysis. Excluded animals were replaced by consecutive experiments until a total of 10 animals in each group were included. The cardioplegic concentrate (1000 mL) was enriched with 5 mL, 10 mg/mL, of esmolol (Brevibloc, Baxter AS, Oslo, Norway) for the intervention group or 5 mL of vehicle for the control group. A total of 0.467 mg/kg (1.6 μmol/kg) of esmolol was administered to pigs in the intervention group. The freshly mixed cardioplegic solution, 12°C, was delivered with a dual-head pump and separate cooling. This will expose the myocardium to concentrations avoiding significant influence from inhibition of L-type Ca^{2+} channels and fast Na^+ channels (Table 1).[18]

Measurements of Cardiac and Hemodynamic Function

Measurements were performed at baseline and 1, 2, and 3 hours after aortic declamping. At each point, arterial blood gases and serum for troponin-T measurements were drawn. Injections of fluorescent microspheres (Dye-Trak "F" ®, Triton Technology Inc., San Diego, CA) were performed with a concurrent sampling of reference arterial blood with a constant rate extraction pump for regional tissue blood flow measurements.[19] General hemodynamics were analyzed with Ponemah Physiology Platform v. 4.90 (Data Sciences International). PV-loop data were exported and analyzed with custom-made software. Hemodynamic variables were averaged over 5-8 consecutive heart beats during a stable situation. The time constant of isovolumetric relaxation, τ, was calculated according to Raff and Glantz.[20] From 6-10 consecutive cardiac cycles during a dynamic preload reduction, load-independent variables were obtained (Fig 1). Systolic function was described by the left ventricular end-systolic pressure-volume relationship (ESPVR) and preload-recruitable stroke work (PRSW). Correspondingly, diastolic compliance was expressed by the linear and the logarithmic end-diastolic pressure-volume relationships (EDPVR$_{lin}$ and β).[21,22] The median correlation coefficient for
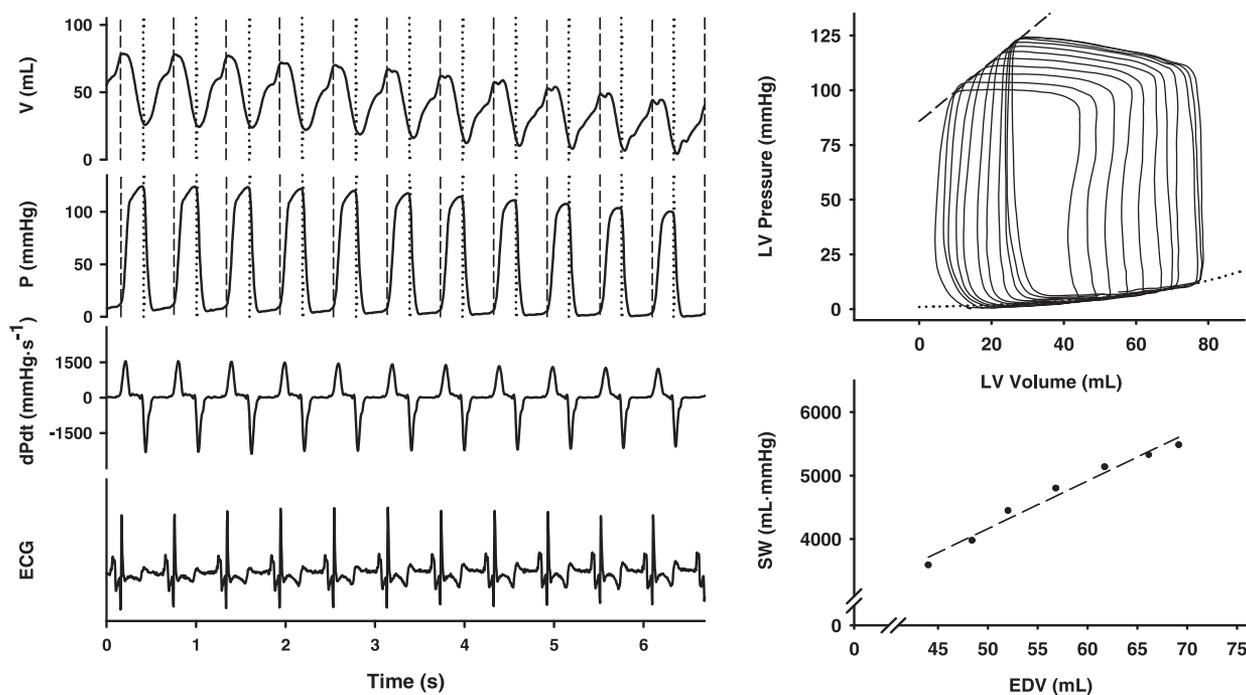
Fig 1. Typical waveforms (left panels) during a dynamic preload reduction recording with end-diastole (dashed lines) and end-systole (dotted line), corresponding pressure-volume loops (upper right panel) with end-systolic pressure-volume relation (dashed line) and logarithmic end-diastolic pressure-volume relation (dotted line) and stroke work as a function of end-diastolic volume with the preload-recruitable stroke work relation (lower right panel). V and P = left ventricular volume and pressure; dP/dt = first derivative of left ventricular pressure; LV = left ventricle; SW = stroke work; EDV = end-diastolic volume.

ESPVR was 0.994 (0.994; 0.982), for PRSW 0.999 (0.999; 0.997), for $EDPVR_{lin}$ 0.989 (0.989; 0.969) and for β 0.986 (0.986; 0.962). Parallel conductance was determined by the hypertonic saline injection method (3 × 5 mL, 10 % NaCl).[23] Variables based on cardiac volumes were normalized for body surface area.[24]

Using a 6-MHz sector probe (6S, GE Vingmed Ultrasound) and a 3 cm thick silicon pad as an offset, an epicardial short-axis view was obtained at the papillary muscle level. By reducing the depth and narrowing the sector, a detailed TDI recording of the left ventricular anterior wall was obtained for radial strain analysis. From an apical 5-chamber long-axis view, including the left ventricular outflow tract and the aortic valve, a pulsed Doppler recording was used to obtain the valve opening and closure, defining start of ejection and end-systole from the Doppler spectrum. All echocardiographic recordings were carried out during respirator shut-off and were optimized for high frame rate. Echocardiographic analyses were performed using EchoPac BT11 (GE Vingmed Ultrasound). Using one cardiac cycle, the short-axis TDI cineloops were analyzed for radial peak systolic strain and radial peak ejection strain rate in 3 manually traced regions of interests (2 × 6 mm, strain length 2 mm) corresponding to the subendocardial, midmyocardial, and epicardial wall layers.[25] Median TDI frame rate was 345 (395; 288) frames/s. During isovolumetric contraction, peak strain rate displayed unphysiologically peak values in a few of the open chest, open pericardium animals.[25] Peak strain rate was, therefore, measured only during the ejection phase.

Tissue and Blood Samples

Immediately after euthanasia, the hearts were removed and tissue samples from the subendocardial, midmyocardial, and subepicardial layers were snap-frozen in liquid nitrogen and later stored at -80°C. Samples were obtained for transmural tissue water content and regional tissue blood flow. The samples used for regional blood flow estimation were hydrolyzed, spheres filtered, colors dissolved, and analyzed on a fluorospectrophotometer as described elsewhere.[19] Myocardial water content was calculated as fraction of wet weight after drying samples for 3 weeks at 60°C.

As markers for apoptotic activity, tissue from all 3 wall layers were analyzed for caspase-3 cleavage by Western blotting (BD-Pharmingen, San Diego, CA), using GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA) to verify equal loading conditions. Caspase-3 activity was determined using the Caspase-3 Colorimetric Assay Kit (BioVision Inc., Milpitas, CA). Tissue was homogenized and lysis performed according to the manufacturer's instructions, and triplet samples containing 400 µg of total protein, measured by the Quick Start Bradford Protein Assay (Bio-Rad, Hercules, CA), were incubated for 2 hours before fluorometric readings. Troponin-T was analyzed according to the hospital clinical routine (Troponin-T hs ®, Roche Diagnostics GmbH, Mannheim, Germany).

Statistical Analysis

Data were analyzed by SPSS v. 20 (IBM, Armonk, NY). Values are given as mean ± SEM or median (75-percentile;

25-percentile) unless otherwise noted. Baseline variables, tissue water content, regional blood flow, caspase-3 activity and troponin-T values at the end of the experiments were analyzed for normal distribution by Kolmogorov-Smirnov tests and equal variance by Levene median tests, and compared with two-sample t-test or Wilcoxon-Mann-Whitney test when appropriate. Variables obtained after declamping were analyzed by two-way analysis of variance for repeated measurements (RM-ANOVA) with time as within factor (p_w) and treatment as grouping factor (p_g). If Mauchly's test of sphericity was significant ($p < 0.05$), the Greenhouse-Geisser adjustment of degrees of freedom was selected. If the level of significance for the interaction between time and group (p_i) was less than 0.1, tests for simple main effects were performed at appropriate factors levels. Cell means finally were compared with Newman-Keuls multiple contrast tests when appropriate. A p value less than 0.05 was considered statistically significant.

RESULTS

Four animals were excluded because of reasons not directly related to technical failure. In one animal from each group, multiple supraventricular arrhythmias made measurements with conductance catheter and echocardiography meaningless. One animal in the esmolol group developed severe pulmonary hypertension followed by pulmonary edema shortly after weaning. One animal in the control group suffered severe arterial hypoxia ($P_aO_2 < 6$ kPa/45 mmHg) and pulmonary hypertension after weaning due to an atrial septal defect found by dissection of the heart.

Characteristics During Baseline and CPB

At baseline, there were no significant differences between groups regarding cardiac and hemodynamic variables (Table 2, Figs 2 and 3). No group differences were found with regard to body weight, rectal temperature, diuresis, arterial blood gases (alpha-stat), troponin-T or respirator settings. Furthermore, there were no significant differences between groups in systemic mean arterial pressure (MAP), rectal temperature or arterial blood gas values during aortic cross-clamping (Table 3).

Cardiovascular Effects Between Groups After Aortic Declamping

Stroke volume decreased gradually in controls after 2 hours ($p < 0.01$) and decreased further ($p < 0.05$) after 3 hours of reperfusion (Table 4). One hour after declamping, cardiac index and the peak positive of the first derivative of left ventricular pressure, dP/dt_{max}, did not differ between groups but decreased in controls to values lower ($p < 0.05$ for both) than in esmolol-treated animals 3 hours after declamping (Fig 2). Ejection fraction (EF) significantly increased in the esmolol group between 1 and 3 hours after declamping, but remained unchanged in controls. PRSW gradually increased in the esmolol group from 1 to 3 hours, with no changes in the control group, demonstrating a group difference at 3 hours after declamping. The relaxation constant, τ, decreased in both groups from 1 to 2 hours ($p < 0.025$) after declamping, further decreasing in the esmolol group only, leading to a significant group difference after 3 hours ($p < 0.025$).

Subendocardial peak systolic strain decreased in the control group and remained unchanged in esmolol-treated animals, with a significant group difference after 3 hours of reperfusion (Fig 3). Peak ejection strain rate in the subendo- and midmyocardial layers increased gradually during the first 3 hours after declamping in esmolol-treated animals as opposed to no difference in the control group. The two study groups differed significantly after 3 hours.

Hemodynamic Changes Over Time Unrelated to Intervention

One hour after declamping no significant differences could be demonstrated between the esmolol and the control group (Table 4, Figs 2 and 3). Heart rate gradually increased in both groups from an average mean value of 103 ± 5 beats/min (n = 20) at 1 hour to 115 ± 7 beats/min ($p < 0.025$) at 2 hours and further to 125 ± 7

Table 2. Baseline Variables Before Cardioplegic Arrest

Variable	Esmolol (n = 10)	Control (n = 10)	Statistics p Value
HR (beats/min)	86 ± 7	85 ± 6	0.88
LV-ESP (mmHg)	107 ± 4	114 ± 8	0.44
LV-ESV$_i$ (mL/m^2)	36 ± 3	34 ± 5	0.76
LV-EDP (mmHg)	8.8 ± 0.9	10.5 ± 1.0	0.22
LV-EDV$_i$ (mL/m^2)	80 ± 4	76 ± 6	0.58
SV$_i$ (mL/m^2)	44 ± 1	42 ± 2	0.37
LV-SWi (mmHg/mL/m^2)	3963 ± 154	3931 ± 235	0.91
LV-dP/dt$_{min}$ (mmHg/s)	-2450 ± 167	-2400 ± 218	0.86
β	0.036 ± 0.004	0.034 ± 0.005	0.81
EDPVR$_{lin}$ (mmHg/mL)	0.16 (0.25 ; 0.15)	0.19 (0.26 ; 0.15)	0.43
MAP (mmHg)	96 ± 5	98 ± 7	0.82
CVP (mmHg)	4.7 ± 0.9	4.2 ± 0.5	0.64
PAP (mmHg)	15 ± 2	15 ± 3	0.99
LV-tissue flow (mL/min/g)	0.92 ± 0.07	0.88 ± 0.10	0.60
RV-tissue flow (mL/min/g)	0.72 ± 0.07	0.61 ± 0.06	0.23

NOTE. Values are mean ± SEM or median (75-percentile; 25-percentile).

Abbreviations: β and EDPVR$_{lin}$, slope of logarithmic and lineary fitted end-diastolic pressure-volume relationship; CVP, mean central venous pressure; dP/dt$_{min}$, peak minimum of first derivative of ventricular pressure; EDP, end-diastolic pressure; EDV, end-diastolic volume; ESP, end-systolic pressure; ESV, end-systolic volume; HR, heart rate; $_i$, value indexed for body surface area; LV, left ventricle; MAP, mean arterial pressure; p, p values from two-sample t-tests or Mann-Whitney Rank Sum Tests; PAP, mean pulmonary artery pressure; RV, right ventricle; SV, stroke volume; SW, stroke work.
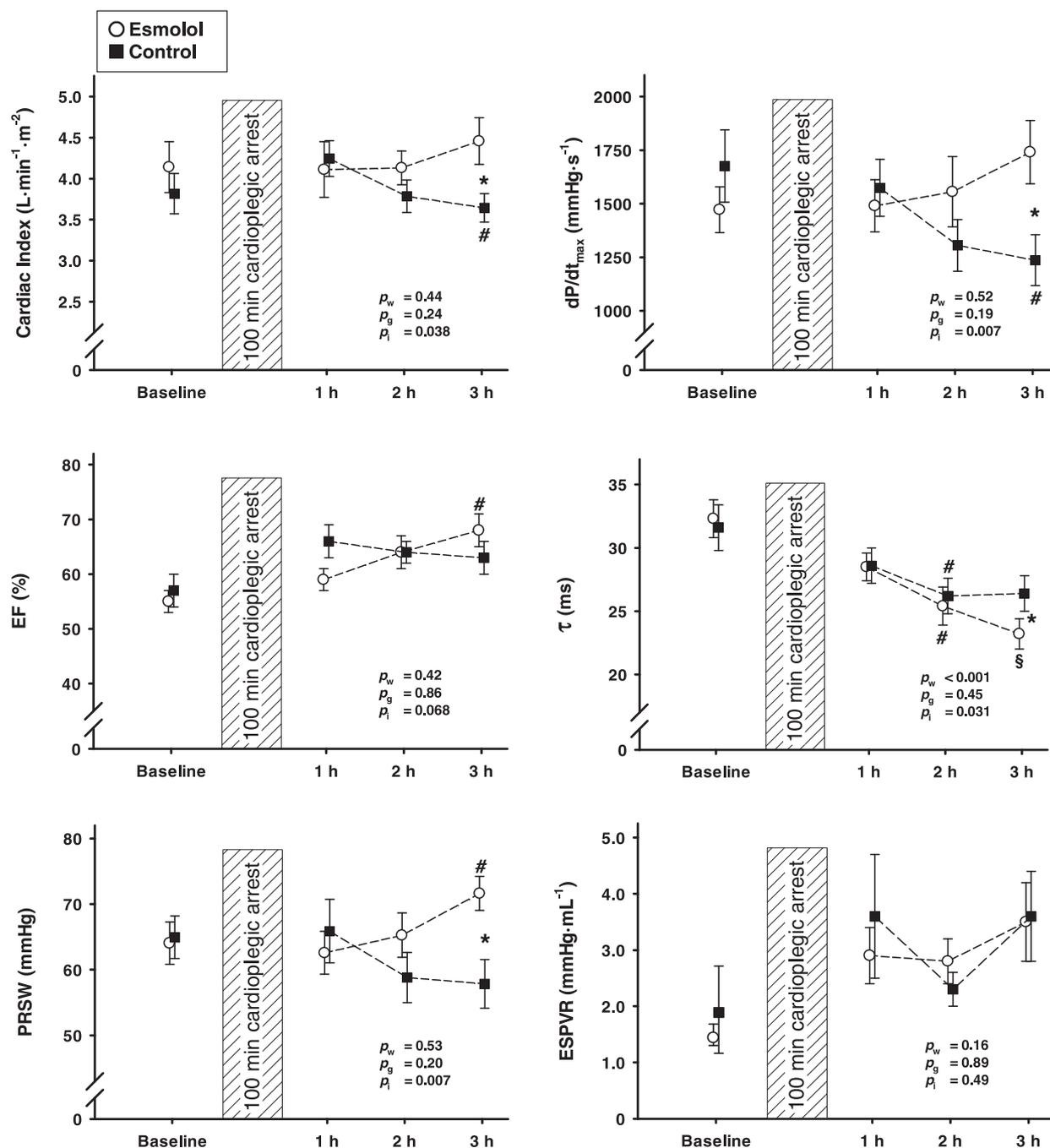
Fig 2. Cardiac Index, peak positive of first derivative of left ventricular pressure (dP/dtmax), ejection fraction (EF), time constant of isovolumetric relaxation (τ), slope of preload-recruitable stroke work (PRSW) and slope of end-systolic pressure-volume relationship (ESPVR) at baseline, 1 hour, 2 hours and 3 hours after aortic declamping. Values are mean $\pm$ SEM or median with quartiles. p_w, p_g, p_i = significance levels within, between groups and for interaction by RM-ANOVA. * = significant difference between group means at 3 hours; § = significantly different from 1 and 2 hours within same group; # = significantly different from 1 hour within same group with Newman-Keuls multiple contrast tests.

beats/min at 3 hours (p < 0.05). Furthermore, there were overall decreases in ventricular pressures and volumes; indexed end-systolic volume decreased from 22 $\pm$ 2 mL/m^2 at 1 hour to 16 $\pm$ 2 mL/m^2 at 3 hours (p < 0.01), whereas the end-systolic pressure and end-diastolic pressure and volume at 1 hour, 85 $\pm$ 4 mmHg, 6.9 $\pm$ 0.4 mmHg and 60 $\pm$ 3 mL/m^2, were significantly reduced already at 2 hours after declamping to 73 $\pm$ 3 mmHg (p < 0.01), 5.7 $\pm$ 0.4

mmHg (p < 0.025) and 52 $\pm$ 3 mL/m^2 (p < 0.001), respectively. The stroke work and peak negative of the first derivative of left ventricular pressure, dP/dtmin, did not differ between groups after reperfusion and decreased from 2,796 $\pm$ 209 mmHg/mL/m^2 at 1 hour to 2,150 $\pm$ 125 mmHg/mL/m^2 after 2 hours for stroke work (p < 0.005) and increased from -1,977 $\pm$ 139 mmHg/s to -1,652 $\pm$ 96 mmHg/s for dP/dtmin (p < 0.01) (n = 20 for all).
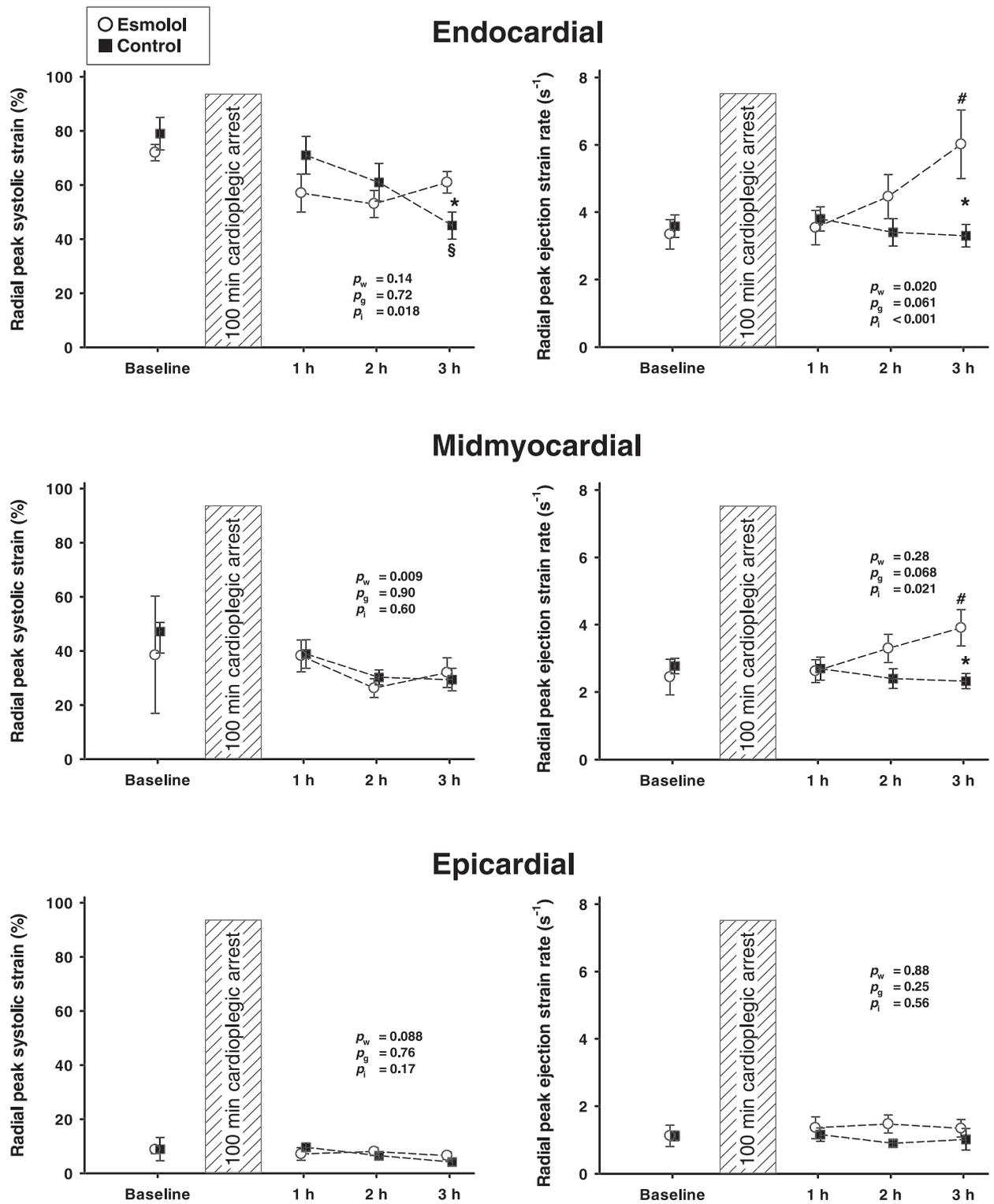
Fig 3. Radial peak systolic strain and radial peak ejection strain rate through left anterior ventricular wall at baseline and 1, 2, and 3 hours after aortic cross-clamp release. Values are mean ± SEM or medians with quartiles. p_w, p_g, p_i = significance levels within, between groups and for interaction by RM-ANOVA. * = significant difference between group means at 3 hours; § = significantly different from 1 and 2 hours within the same group; # = significantly different from 1 hour within same group with Newman-Keuls multiple contrast tests.

Table 3. Mean Arterial Pressure, Temperature, Hemoglobin and Arterial Blood Gases During Cardiopulmonary Bypass and Aortic Clamp in 2 Groups of Pigs With 100 Minutes of Cardioplegic Arrest

Variable	2 min before X-clamp	50 min X-clamp	98 min X-Clamp	Statistics
MAP (mmHg)				
Esmolol	78 ± 4	51 ± 3	64 ± 4	$p_w < 0.001$, $p_g = 0.92$, $p_i = 0.59$
Control	83 ± 8	49 ± 6	60 ± 4	
Temp$_{rect}$ (°C)				
Esmolol	38.6 ± 0.3	34.9 ± 0.1	37.8 ± 0.1	$p_w < 0.001$, $p_g = 0.24$, $p_i = 0.79$
Control	38.9 ± 0.3	35.0 ± 0.1	38.2 ± 0.3	
Hb (g/dL)				
Esmolol	7.6 ± 0.2	8.6 ± 0.4	8.9 ± 0.2	$p_w < 0.001$, $p_g = 0.95$, $p_i = 0.50$
Control	7.6 ± 0.2	8.8 ± 0.2	8.8 ± 0.3	
pH				
Esmolol	7.44 ± 0.01	7.42 ± 0.01	7.43 ± 0.02	$p_w = 0.23$, $p_g = 0.94$, $p_i = 0.63$
Control	7.43 ± 0.01	7.41 ± 0.01	7.44 ± 0.01	
pO$_2$ (kPa)				
Esmolol	14.3 ± 1.5	17.0 ± 1.1	14.7 ± 1.2	$p_w = 0.86$, $p_g = 0.70$, $p_i = 0.13$
Control	16.2 ± 2.1	14.3 ± 0.9	16.9 ± 0.6	
pCO$_2$ (kPa)				
Esmolol	5.5 ± 0.1	6.1 ± 0.2	5.6 ± 0.3	$p_w = 0.052$, $p_g = 0.57$, $p_i = 0.99$
Control	5.7 ± 0.2	6.1 ± 0.2	5.7 ± 0.2	
HCO$_3$ (mmol/L)				
Esmolol	27.5 ± 0.6	28.8 ± 0.6	28.2 ± 0.6	$p_w = 0.21$, $p_g = 0.67$, $p_i = 0.56$
Control	27.9 ± 0.4	28.5 ± 0.4	28.9 ± 1.2	
s-K$^+$ (mmol/L)				
Esmolol	4.5 ± 0.1	4.9 ± 0.1	5.6 ± 0.1	$p_w < 0.001$, $p_g = 0.36$, $p_i = 0.33$
Control	4.4 ± 0.1	4.9 ± 0.1	5.4 ± 0.1	
s-Na$^+$ (mmol/L)				
Esmolol	139 ± 1	138 ± 1	138 ± 1	$p_w = 0.21$, $p_g = 0.39$, $p_i = 0.73$
Control	140 ± 1	139 ± 1	138 ± 2	

NOTE. Values are mean $\pm$ SEM, n = 10.

Abbreviations: MAP, mean aortic pressure; p_g, p value for between groups from two-way RM-ANOVA; p_i, p value for interaction from two-way RM-ANOVA; p_w, p values for within subjects from two-way RM-ANOVA; RM-ANOVA, repeated-measures analysis of variance; s, serum levels; Temp$_{rect}$, rectal temperature.

The logarithmic fitted end-diastolic pressure-volume relationship, β, increased in both groups from 1 to 2 hours (p < 0.001) and further increased at 3 hours (p < 0.01) with no significant differences between groups.

Tissue and Blood Samples

Arterial blood gases were within physiologic ranges during reperfusion with no differences between groups. Myocardial tissue water content in esmolol-treated animals was 80.4 ± 0.1% compared to controls 80.8 % ± 0.1 (p = 0.04). There were no significant differences in myocardial blood flow between groups in left and right ventricular tissue measured by microspheres between groups 1, 2, and 3 hours after reperfusion (Table 4). The level of cleaved caspase-3 was lower in the left ventricular subendocardial wall layer in the esmolol group compared to controls (p = 0.02) (Fig 4). However, caspase-3 activity did not show any significant difference between groups in wall layers. Three hours after aortic declamping, troponin-T was 339 ± 48 ng/L for the esmolol group and 357 ± 55 ng/L for the control group (p = 0.81).

DISCUSSION

The main finding in this study was that the addition of esmolol to repeated, cold, oxygenated blood cardioplegic solution preserves the left ventricular contractile function in this clinically relevant animal model with 100 minutes of cardioplegic arrest and 3 hours of reperfusion.

Both EF and radial peak systolic TDI strain in the subendocardium were better preserved 3 hours after declamping in the esmolol group compared to the control group. These variables, describing left ventricular systolic function, are both pre- and afterload dependent.[26] However, LV-EDV$_i$, LV-EDP, and MAP did not differ between groups, demonstrating comparable loading conditions. Furthermore, in the esmolol-treated animals, there was an increased dP/dt$_{max}$ as well as an increased subendocardial and midmyocardial strain rate 3 hours after declamping (Figs 2 and 3). These variables are more load-independent compared to strain.[26] Also, in the esmolol-treated animals the load-independent variable PRSW clearly increased from 1 to 3 hours after declamping and weaning to levels significantly higher at 3 hours compared to controls. Together with an overall better-preserved cardiac index, all of these findings demonstrated increased contractility when esmolol is added to the cardioplegic solution. The slope of ESPVR, however, did not show a similar pattern. One explanation for this could be that at low ventricular pressures and volumes this relation is often nonlinear, making the forced fitting of a linear model more inaccurate.[22] This also is reflected by the variability of ESPVR in the present study.

The preserved contractile function in the esmolol group is consistent with a more sensible adrenergic receptors apparatus. During CPB a stress response with a corresponding rise in serum catecholamines occur.[27,28] In addition, endogenous norepinephrine may be released in the myocardium as a response to anoxia and cooling during cardioplegic arrest.[11-13] Furthermore, this adrenergic

Table 4. Cardiac and Hemodynamic Variables and Tissue Blood Flow 1, 2, and 3 Hours After Aortic Declamping

Variable	1 hour	2 hours	3 hours	Statistics
HR (beat/min)				
Esmolol	107 ± 6	123 ± 9	130 ± 10	$p_w = 0.001$, $p_g = 0.33$, $p_i = 0.66$
Control	98 ± 8	107 ± 9	119 ± 11	
LV-ESP (mmHg)				
Esmolol	87 ± 5	69 ± 2	70 ± 3	$p_w = 0.001$, $p_g = 0.56$, $p_i = 0.34$
Control	84 ± 6	76 ± 4	72 ± 3	
LV-ESV$_i$ (mL/m^2)				
Esmolol	24 ± 3	19 ± 3	15 ± 2	$p_w = 0.010$, $p_g = 0.33$, $p_i = 0.50$
Control	22 ± 3	20 ± 3	17 ± 2	
LV-EDP (mmHg)				
Esmolol	7.4 ± 0.7	5.6 ± 0.7	5.9 ± 0.8	$p_w = 0.011$, $p_g = 0.70$, $p_i = 0.19$
Control	6.3 ± 0.7	5.9 ± 0.6	5.6 ± 0.8	
LV-EDV$_i$ (mL/m^2)				
Esmolol	59 ± 4	51 ± 4	46 ± 3	$p_w < 0.001$, $p_g = 0.64$, $p_i = 0.92$
Control	62 ± 4	53 ± 4	47 ± 4	
SV$_i$ (mL/m^2)				
Esmolol	34 ± 3	31 ± 3	30 ± 2	$p_w < 0.001$, $p_g = 0.50$, $p_i = 0.077$
Control	41 ± 3	33 ± 2[*]	29 ± 2[*†]	
LV-SW$_i$ (mmHg/mL/m^2)				
Esmolol	2691 ± 304	2049 ± 176	2079 ± 168	$p_w = 0.001$, $p_g = 0.90$, $p_i = 0.22$
Control	2902 ± 299	2251 ± 182	1757 ± 146	
LV-dP/dt$_{min}$ (mmHg/s)				
Esmolol	-1954 ± 206	-1630 ± 131	-1685 ± 126	$p_w = 0.002$, $p_g = 0.94$, $p_i = 0.43$
Control	-1999 ± 199	-1674 ± 146	-1554 ± 106	
β (unit-less)				
Esmolol	0.034 ± 0.004	0.052 ± 0.010	0.062 ± 0.010	$p_w = 0.005$, $p_g = 0.39$, $p_i = 0.14$
Control	0.038 ± 0.005	0.038 ± 0.005	0.047 ± 0.010	
EDPVR$_{lin}$ (mmHg/mL)				
Esmolol	0.16 ± 0.03	0.16 ± 0.02	0.21 ± 0.03	$p_w = 0.018$, $p_g = 0.31$, $p_i = 0.60$
Control	0.14 ± 0.01	0.14 ± 0.02	0.17 ± 0.02	
MAP (mmHg)				
Esmolol	72 ± 6	56 ± 1	53 ± 2	$p_w < 0.001$, $p_g = 0.26$, $p_i = 0.67$
Control	79 ± 7	63 ± 4	57 ± 3	
CVP (mmHg)				
Esmolol	5.0 ± 0.7	4.3 ± 0.7	4.4 ± 0.7	$p_w < 0.001$, $p_g = 0.50$, $p_i = 0.42$
Control	5.9 ± 0.9	4.8 ± 0.9	5.3 ± 0.9	
PAP (mmHg)				
Esmolol	20 ± 1	17 ± 1	18 ± 1	$p_w = 0.69$, $p_g = 0.40$, $p_i = 0.86$
Control	18 ± 1	16 ± 1	16 ± 2	
LV-tissue flow (mL/min/g)				
Esmolol	1.17 ± 0.10	1.11 ± 0.09	1.18 ± 0.08	$p_w = 0.13$, $p_g = 0.10$, $p_i = 0.31$
Control	1.08 ± 0.08	0.91 ± 0.07	0.94 ± 0.08	
RV-tissue flow (mL/min/g)				
Esmolol	1.17 ± 0.14	1.07 ± 0.13	1.25 ± 0.11	$p_w = 0.016$, $p_g = 0.23$, $p_i = 0.42$
Control	1.08 ± 0.07	0.89 ± 0.07	1.03 ± 0.08	

NOTE. Values are mean ± SEM.

Abbreviations: β and EDPVR$_{lin}$, slope of logarithmic and linearly fitted end-diastolic pressure-volume relationship; CVP, mean central venous pressure; dP/dt$_{min}$, peak minimum of first derivative of ventricular pressure; EDP, end-diastolic pressure; EDV, end-diastolic volume; ESP, end-systolic pressure; ESV, end-systolic volume; HR, heart rate; $_i$, value indexed for body surface area; LV, left ventricle; MAP, mean arterial pressure; PAP, mean pulmonary artery pressure; p_g, p value for between groups from two-way RM-ANOVA; p_i, p values for interaction from two-way RM-ANOVA; p_w, p values for within subjects from two-way RM-ANOVA; RM-ANOVA, repeated-measures analysis of variance; RV, right ventricle; SV, stroke volume; SW, stroke work.

*Significant difference within group means from 1 hour.

†Significant difference within group means from 2 hours.

stimulation triggers desensitization of β-adrenergic receptors via G-protein uncoupling as shown in both animal experiments as well as in clinical studies.[29,30] Esmolol has been shown to alleviate β-adrenergic receptor desensitization when given as a continuous infusion during CPB.[10] This also may explain the more rapid isovolumetric relaxation observed by reduction of τ in the esmolol group, because the isovolumetric relaxation is enhanced by β-adrenergic stimulation via mobilization of cytosolic Ca^{2+}.

Myocardial tissue blood flow did not differ between groups. However, there was a tendency of decreasing blood flow in the control group. This is in agreement with the differences seen in systolic function. Therefore, an enhanced adrenergic response
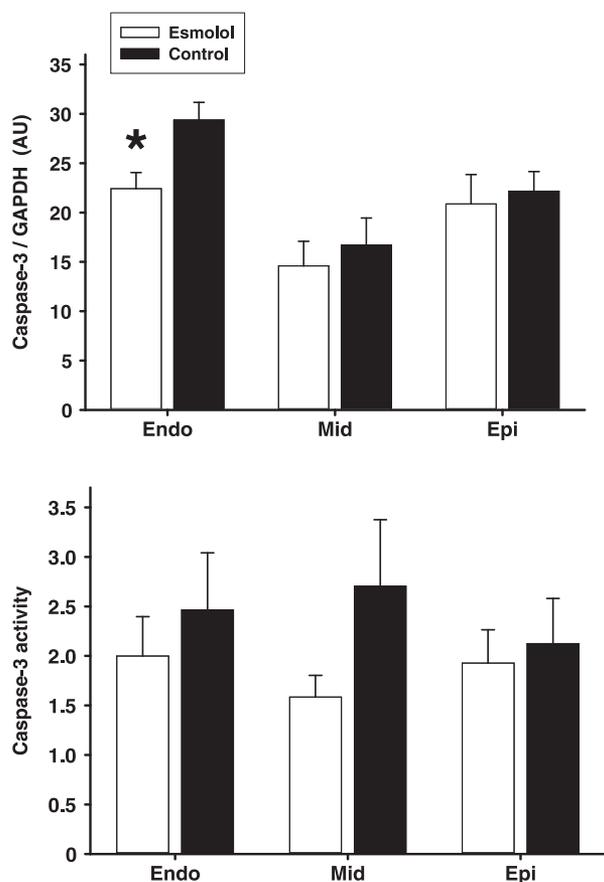
Fig 4. Cleaved caspase-3 relative to GAPDH and caspase-3 activity trough the left ventricular myocardial wall layers 3 hours after aortic declamping. Bars are mean + SEM. * = significant difference between groups by two-sample t-test.

to the decline in loading conditions rather than alleviated myocardial stunning could explain the hemodynamic differences between groups.

A modest amount of esmolol added to an existing cardioplegic regimen, may be an appealing strategy to utilize the potential protective effects of β-adrenergic receptor blocking agents. Experimentally, esmolol administered as pretreatment or at declamping improves cardiac function after CPB and cardioplegic arrest.[8–10] However, in these studies the myocardium only is exposed to esmolol for a limited period of time. By adding esmolol to the cardioplegic perfusate, the authors aimed at exposing the myocardium during the entire cross-clamp time, counteracting both potential ischemic and lethal reperfusion injury and desensitization of β-adrenergic receptors by endogenous catecholamine release. The half-life for esmolol in blood is only 9 minutes because of the rapid degradation with blood esterases.[31] Consequently, the cardioplegic solution must be administered with a dual-head pump mixing blood and the cardioplegic concentrate at the time of delivery. The route of administration also allowed exposing the myocardium to relatively high doses of esmolol but only adding up to low systemic concentrations. This, in combination with the fact that the final cardioplegic perfusion was completed 18 minutes before aortic declamping, should minimize the danger of significant accumulation of esmolol during weaning

that could hamper cardiac performance in the early postoperative phase.

A small but significant difference in cleaved caspase-3 levels in the subendocardial wall layer of esmolol-treated animals indicated some degree of protection against apoptosis. This may be initiated either by ischemia or by lethal reperfusion injury.[32] However, no significant differences between groups were found in troponin-T release 3 hours after declamping. This contradicts that the addition of esmolol to the cardioplegic solution reduced reperfusion induced injury.

The small but significant difference in myocardial water content between groups observed in the current study agreed with several studies showing strong correlations between myocardial water content and the reduction in myocardial contractility, increased isovolumetric relaxation and increased diastolic stiffness.[33–35] The mechanism underlying this correlation is not understood clearly, but altered compliance and reduced diffusion because of an expansion in the interstitium have been suggested.[36]

It should be noted that high concentrations of esmolol may alter isolated myocyte membrane potentials through blocking of L-type Ca^{2+} channels and fast Na^+ channels, resulting in direct negative inotropic effects beyond the β-adrenergic receptor antagonism.[18,37] In the present study, the myocardium was exposed to relatively high concentrations of esmolol. During the high-dose cardioplegic perfusion (Table 1) the calculated concentration of esmolol in the freshly mixed cardioplegic solution was 34 μM. This was just within the margins of what also may contribute to blocking of Na^+ and Ca^{2+} channels in myocytes, which in turn could contribute to keep the myocardial membrane potential closer to resting values, potentially enhancing protective effects of the cardioplegia.

Limitations

This study was performed using a porcine model with young, healthy pigs and clinical interpretations should be carried out with care. Relatively few animals were studied, but with a strict and standardized protocol to obtain maximal statistical power. Smaller differences and more biologic variance could be expected in a clinical setting including individual supportive treatment. For both groups of pigs, a gradual decay in cardiovascular loading conditions after weaning was indicated by the reductions in systemic and left ventricular pressures, left ventricular volumes, and the increase in heart rate. No individual vasoactive support beyond the standardized continuous saline infusion was allowed in the protocol. Also, from previous experience the authors knew that the current model would deteriorate over time, which was the reason for only 3 hours of observation time after aortic declamping. On the other hand, the cardiac response to these altered loading conditions very well may have accentuated the difference in contractile response and, thus, revealed the difference between the 2 groups.

CONCLUSION

The current study demonstrated that the addition of esmolol to repeated, cold, oxygenated blood cardioplegia preserved systolic function the first 3 hours after aortic declamping in a porcine model with 100 minutes of cardioplegic arrest. The study can contribute to the attempts to improve and optimize the myocardial protection

properties of repeated, cold, oxygenated blood cardioplegia. The technique for administering cardioplegia with esmolol used in the present study does not require any additional instrumentation or time, and easily could be adapted in a clinical trial and eventually also into clinical practice.

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