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Myocardial energy metabolism and ultrastructure with polarizing and depolarizing cardioplegia in a porcine model[†]

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Abstract

OBJECTIVES: This study investigated whether the novel St. Thomas' Hospital polarizing cardioplegic solution (STH-POL) with esmolol/adenosine/magnesium offers improved myocardial protection by reducing demands for high-energy phosphates during cardiac arrest compared to the depolarizing St. Thomas' Hospital cardioplegic solution No 2 (STH-2).

METHODS: Twenty anaesthetised pigs on tepid cardiopulmonary bypass were randomized to cardiac arrest for 60 min with antegrade freshly mixed, repeated, cold, oxygenated STH-POL or STH-2 blood cardioplegia every 20 min. Haemodynamic variables were continuously recorded. Left ventricular biopsies, snap-frozen in liquid nitrogen or fixed in glutaraldehyde, were obtained at Baseline, 58 min after cross-clamp and 20 and 180 min after weaning from bypass. Adenine nucleotides were evaluated by high-performance liquid chromatography, myocardial ultrastructure with morphometry.

RESULTS: With STH-POL myocardial creatine phosphate was increased compared to STH-2 at 58 min of cross-clamp [59.9 ± 6.4 (SEM) vs 44.5 ± 7.4 nmol/mg protein; $P < 0.025$], and at 20 min after reperfusion (61.0 ± 6.7 vs 49.0 ± 5.5 nmol/mg protein; $P < 0.05$), ATP levels were increased at 20 min of reperfusion with STH-POL (35.4 ± 1.1 vs 32.4 ± 1.2 nmol/mg protein; $P < 0.05$). Mitochondrial surface-to-volume ratio was decreased with polarizing compared to depolarizing cardioplegia 20 min after reperfusion (6.74 ± 0.14 vs $7.46 \pm 0.13 \mu\text{m}^2/\mu\text{m}^3$; $P = 0.047$). None of these differences were present at 180 min of reperfusion. From 150 min of reperfusion and onwards, cardiac index was increased with STH-POL; 4.8 ± 0.2 compared to 4.0 ± 0.2 l/min/m² ($P = 0.011$) for STH-2 at 180 min.

CONCLUSIONS: Polarizing STH-POL cardioplegia improved energy status compared to standard STH-2 depolarizing blood cardioplegia during cardioplegic arrest and early after reperfusion.

Keywords: Cardioplegia • Myocardial protection • Energy metabolism • Ultrastructure

INTRODUCTION

Repeated hyperkalaemic blood cardioplegia, considered by many to provide optimal myocardial protection, induces depolarization and re-equilibration of the resting cell membrane potential. This inactivates the voltage-dependent fast Na⁺ channels [1], prevents initiation of the action potential and induces depolarized diastolic arrest. However, this will cause intracellular Na⁺ and Ca²⁺ overload by activation of the Na⁺/H⁺-exchanger and reversal of the Na⁺/Ca²⁺ exchanger; the resulting imbalance in cytosolic ion composition activates energy utilizing compensatory mechanisms [2].

By inducing 'polarized' (non-depolarized) arrest the myocyte membrane potential is maintained at or near the resting membrane potential [3]. In isolated hearts, polarized cardiac arrest reduces myocardial oxygen consumption compared to depolarized arrest, probably as a result of reduced activation of energy-dependent cellular ion channels/pumps, and hence further reduced needs for high-energy phosphates (HEP) during cardiac arrest [4]. The normokalaemic St. Thomas' Hospital polarizing cardioplegic solution (STH-POL) with esmolol/adenosine/magnesium avoids depolarized arrest and reduces the potassium load. A recent experimental study demonstrated comparable myocardial protection and improved postoperative contractility with STH-POL cardioplegia compared to St. Thomas' Hospital cardioplegic

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solution No 2 (STH-2) [5]. In the present study, using the same clinically relevant porcine model, we investigated whether cardioplegic arrest with STH-POL offers myocardial protection comparable to a standard depolarizing potassium-based cardioplegic solution (STH-2), with the main focus on tissue levels of HEP and myocardial ultrastructure during aortic clamping and early and late after weaning from cardiopulmonary bypass (CPB).

METHODS

Anaesthesia and instrumentation

The experiments were conducted in accordance with the European Communities Council Directive of 2010 (63/EU) and approved by the Norwegian State Commission for Laboratory Animals (Project 20135835). Twenty-two pigs (Norwegian land race) of either gender, weighing 42 ± 3 (SD) kg were premedicated with an i.m. injection of ketamine, diazepam and atropine. Spontaneous ventilation on mask with oxygen and 3% isoflurane (Rhodia, Bristol, UK) allowed intravenous access through two ear veins. Loading doses followed by continuous infusions of fentanyl, midazolam, pentobarbital and pancuronium secured general anaesthesia and analgesia. This anaesthetic protocol has previously been described and thoroughly evaluated allowing the safe use of neuromuscular blocking agents in young pigs [5, 6]. The animals were tracheotomized, intubated and ventilated (Julian, Drägerwerk, Lübeck, Germany) with nitrous oxide (56–58%) and oxygen. Fluid substitution was Ringers acetate (15 ml/kg/h) with added KCl (20 mmol/l) to maintain normal plasma potassium levels after overnight fasting. In addition, Ringers acetate 5 ml/kg/h was provided after weaning from CPB.

The right femoral artery and vein were cannulated for blood sampling and infusion. An arterial blood gas analysis determined the need for ventilator adjustments (ABL80 FLEX COOX, Radiometer Medical ApS, Brønshøj, Denmark). A surgically inserted catheter measured diuresis and rectal temperature was monitored. A midline sternotomy and pericardiotomy exposed the heart and heparin (125 IU/kg) was given i.v. to prevent catheter clotting. A continuous cardiac output catheter (CCO/EDV 177HF 75, Edwards Lifesciences Inc., Irvine, CA) was floated into the pulmonary artery via the left internal mammary vein. Cardiac output, right ventricular end-diastolic volume, central venous pressure (CVP) and pulmonary artery pressure (PAP) (Vigilance II® and TruWave® transducers, Edwards Lifescience Inc.) were monitored. The proximal aorta was cannulated with a Millar microtip pressure catheter (Millar MPC-500, Houston, TX, USA) through the left internal mammary artery. An identical catheter through the apex of the heart monitored left ventricular pressure. The haemodynamic parameters were recorded by a 16-channel Ponemah (ACQ-7700; Data Sciences International, St. Paul, MN, USA).

Cardiopulmonary bypass and cardioplegia

The heart-lung machine (Stöckert SIII, Munich, Germany) was primed with 1200 ml Ringers acetate. Heparin 500 IU/kg i.v. was given before the brachiocephalic artery (EOPA 18 Fr, Medtronic Inc., Minneapolis, MN, USA) and right atrial appendage (MC2 28/36 Fr, Medtronic Inc.) were cannulated and CPB established with a flow of 90 ml/min per kg and water temperature of 32°C in the heat exchanger. Aortic cross-clamp time was 60 min and a vent

catheter (DLP 13, Medtronic Inc.) temporarily placed through the apex for left ventricular drainage. The body temperature was allowed to drift and CPB flow was reduced to 72 ml/min per kg when rectal temperature reached 35°C or after 20 min. After 40 min of cross-clamping, rewarming was initiated with reset of CPB-flow to 90 ml/min per kg and water temperature at 40°C. Arterial blood gases were obtained before aortic cross-clamping and just before declamping after 60 min. Defibrillation of ventricular fibrillation was the only allowed antiarrhythmic intervention. After 10 min of reperfusion the animals were weaned from CPB and decannulated. The residual blood in the circuit was returned and protamine sulphate 1.5 mg/kg was given.

Cardioplegia was given as either the hyperkalaemic St. Thomas' Hospital cardioplegic solution 2 (STH-2), or the normokalaemic St. Thomas' Hospital polarizing solution (STH-POL). Both solutions were pre-prepared as concentrate and administered as cold (12°C), oxygenated, blood cardioplegia, freshly mixed by a dual-head pump and separate cooling. The cardioplegia was delivered into the aortic root with a flow set to 7% of CPB-flow, following a standardized protocol with an initial 'high-dose' (1:4 concentrate/blood) for 3 min and 2 min of 'low-dose' (1:8 concentrate/blood) given at 20 and 40 min after cross-clamping. The final concentrations of key components in the cardioplegic solutions are presented in the Supplementary Material, Table A.

Experimental protocol

The animals were block-randomized to the STH-POL or STH-2 group (10 per group). After 10 min of stabilization Baseline arterial blood gases and haemodynamic variables were recorded. Two myocardial biopsies from the anterior left ventricular wall were obtained by using 6 and 4 mm biopsy punch (Integra Miltex, York, PA, USA). Both samples included tissue from the epi- and midmyocardium; the 6-mm biopsy was snap-frozen in liquid nitrogen within 5 s and stored at -80°C for later analysis while the 4-mm biopsy was cut and fixed directly in glutaraldehyde.

During CPB the ventilator volume was reduced to 50% and with passive drainage of the left ventricle. Additional heparin (250 IU/kg) was given at 30 min of cross-clamping. After 58 min of cardioplegic arrest, myocardial biopsies were obtained and 2 min later the aortic clamp was removed. The animals were weaned from CPB after 10 min of myocardial reperfusion and decannulated. Twenty and 180 min after declamping myocardial biopsies were taken similar to the Baseline situation. General haemodynamics were recorded every 15 min, from 30 to 180 min after declamping. Finally, the animal was euthanized with intracardiac injection of high dose potassium chloride, the heart removed and samples obtained for complimentary analysis.

Myocardial metabolism and ultrastructure

High energy phosphates [ATP, ADP, AMP and creatine phosphate (CrP)], hypoxanthine and xanthine were measured by high-performance liquid chromatography analysis as previously described [7]. Energy charge (EC) was calculated as:

$$EC = \frac{[ATP] + 0.5 * [ADP]}{[ATP] + [ADP] + [AMP]}$$

Myocardial volume fraction of mitochondria, myofibrils and cytosol were quantified by point counting in 6 randomly selected micrographs ($\times 15\,000$) from each biopsy, mitochondrial surface

to volume ratio and surface density were calculated by using a Merz grid and the freeware ImageJ [8]. To evaluate inter- and intra-observer variability, the 6 micrographs from 15 randomly selected biopsies were recorded and analysed individually by two observers and by one of the observers twice. For details see Supplementary Material.

Statistical analysis

Data were analysed using SPSS v. 23 (IBM Corp., Armonk, NY, USA) and values given as mean \pm SEM or median (25% percentile; 75% percentile) unless otherwise noted. At Baseline groups were compared by two-sample Student *t*-test on data with normal distribution and with Wilcoxon-Mann-Whitney test on ranks whenever appropriate. Tissue contents of HEPs, degradation products and haemodynamic variables after aortic declamping were compared with two-way ANOVA for repeated measurements (RM-ANOVA) with treatment as group factor (P_g), time as within factor (P_w) and P_i for interaction between time and group. Morphological variables were analysed with nested RM-ANOVA. $P < 0.05$ was considered statistically significant. The intraclass correlation coefficient (ICC) and coefficient of variation (CV%) for inter- and intra-observer variability were calculated for morphometric data. For details see Supplementary Material.

RESULTS

Two animals were excluded. After declamping, one animal in the STH-POL group with profound bleeding and ventricular fibrillation prior to cross-clamp developed severe left heart failure, one animal in the STH-2 group developed severe pulmonary hypertension and right heart failure. Excluded animals were replaced by consecutive experiments. Results are given for 10 animals in each group, except for morphometry where the biopsies from one STH-2 animal were lost. Haemodynamic variables, left ventricular function and arterial blood gases did not differ between groups at Baseline (Fig. 1A–F and Table 1). After 58 min of cardioplegic arrest, the level of serum potassium was significantly increased in the STH-2 compared to the STH-POL group (5.2 ± 0.3 vs 4.1 ± 0.1 mmol/l, $P < 0.001$). During CPB there were no other differences between groups regarding mean arterial pressure, temperatures and arterial blood gases (Supplementary Material, Table B).

Cardiac and haemodynamic variables after weaning

Heart rate increased over time in both groups after weaning (Fig. 1A). In both groups, cardiac index (CI) was initially increased from Baseline but subsequently decreased to a plateau level by 90 min; there were no differences between groups during this period. At 150, 165 and 180 min after declamping, CI was increased in the STH-POL compared to the STH-2 group ($P = 0.036$, $P = 0.026$ and $P = 0.011$, respectively) (Fig. 1B). Left ventricular systolic pressure was unchanged and left ventricular end-diastolic pressure decreased gradually after declamping in both groups (Fig. 1C and D). The first derivative of the left ventricular pressure, $LV-dP/dt_{max}$, did not change significantly over time (Fig. 1E). $LV-dP/dt_{min}$ was less negative in the STH-POL group after 30 min ($P = 0.006$) and 45 min ($P = 0.048$) of reperfusion

compared to corresponding values in the STH-2 group (Fig. 1F). Right ventricular indexed end-diastolic volume (RV-EDV_i) and ejection fraction (RV-EF) did not change over time (Table 2). Indexed stroke volume (RV-SV_i) decreased from 60 to 120 min of reperfusion ($P < 0.001$) for both groups. Central venous pressure (CVP) gradually increased over time ($P_w = 0.030$) in both groups. There were no significant differences between groups regarding mean arterial pressure and pulmonary arterial pressure.

Myocardial high-energy phosphates

At Baseline there were no significant differences between groups for myocardial CrP, adenine nucleotides and the degradation products hypoxanthine and xanthine (Fig. 2A–F). CrP was significantly increased in hearts arrested with STH-POL compared to STH-2 after 58 min of cardiac arrest (59.9 ± 6.4 vs 44.5 ± 7.4 nmol/mg protein; $P < 0.025$) and after 20 min of reperfusion (61.0 ± 6.7 vs 49.0 ± 5.5 nmol/mg protein; $P < 0.05$). However, at 180 min after declamping, the CrP content did not differ between groups (Fig. 2A). At 20 min of reperfusion tissue ATP content was increased in the STH-POL compared to the STH-2 group ($P < 0.05$) (Fig. 2B). Tissue levels of ADP increased from Baseline to 58 min after cardiac arrest in both groups ($P < 0.001$) and decreased to Baseline levels ($P < 0.001$) after 20 and 180 min of reperfusion (Fig. 2C). AMP levels increased in both groups from Baseline to 58 min of cardiac arrest ($P < 0.01$) and did not decrease after reperfusion (Fig. 2D). The level of hypoxanthine increased significantly ($P < 0.001$) in the STH-POL group, and remained increased at the same level at 20 min after declamping (Fig. 2E). After 180 min hypoxanthine levels decreased to Baseline levels ($P < 0.001$). Xanthine levels did not differ between groups and did not change with time (Fig. 2F). There was an overall change in energy charge (EC) over time ($P_w = 0.025$) with no group differences (Table 3). However, multiple contrast tests could not confirm differences between individual time points. The ATP/ADP ratio (pooled means, $n = 20$) decreased from Baseline to 58 min of cross-clamping ($P < 0.001$) and returned to Baseline levels after 20 and 180 min of reperfusion. The CrP/ATP ratio was unchanged.

Mitochondrial ultrastructure

Mitochondrial surface-to-volume ratio ($S_{vratio_{mit}}$) was slightly decreased in the STH-POL compared to the STH-2 group at 20 min after reperfusion (6.74 ± 0.14 vs 7.46 ± 0.13 $\mu\text{m}^2/\mu\text{m}^3$; $P = 0.047$) (Fig. 3A). None of these differences were present at 180 min of reperfusion. Neither mitochondrial surface density (S_{vmit}) nor the myocyte volume fractions of mitochondria, myofibrils and cytosol differed between groups (Fig. 3B and Table 4). For the fractional volumes the interobserver ICC varied between 0.741 and 0.897 with a CV% between 2.48% for myofibrils and 8.14% for sarcoplasm (Table 5). For mitochondrial surface to volume ratio the interobserver ICC was 0.790 with a CV% of 4.05%. Based on ICC calculations, both the inter- and intra-observer variabilities for morphometric data were classified as good to excellent, except for the intra-observer ICC for volume fraction of sarcoplasm [9].

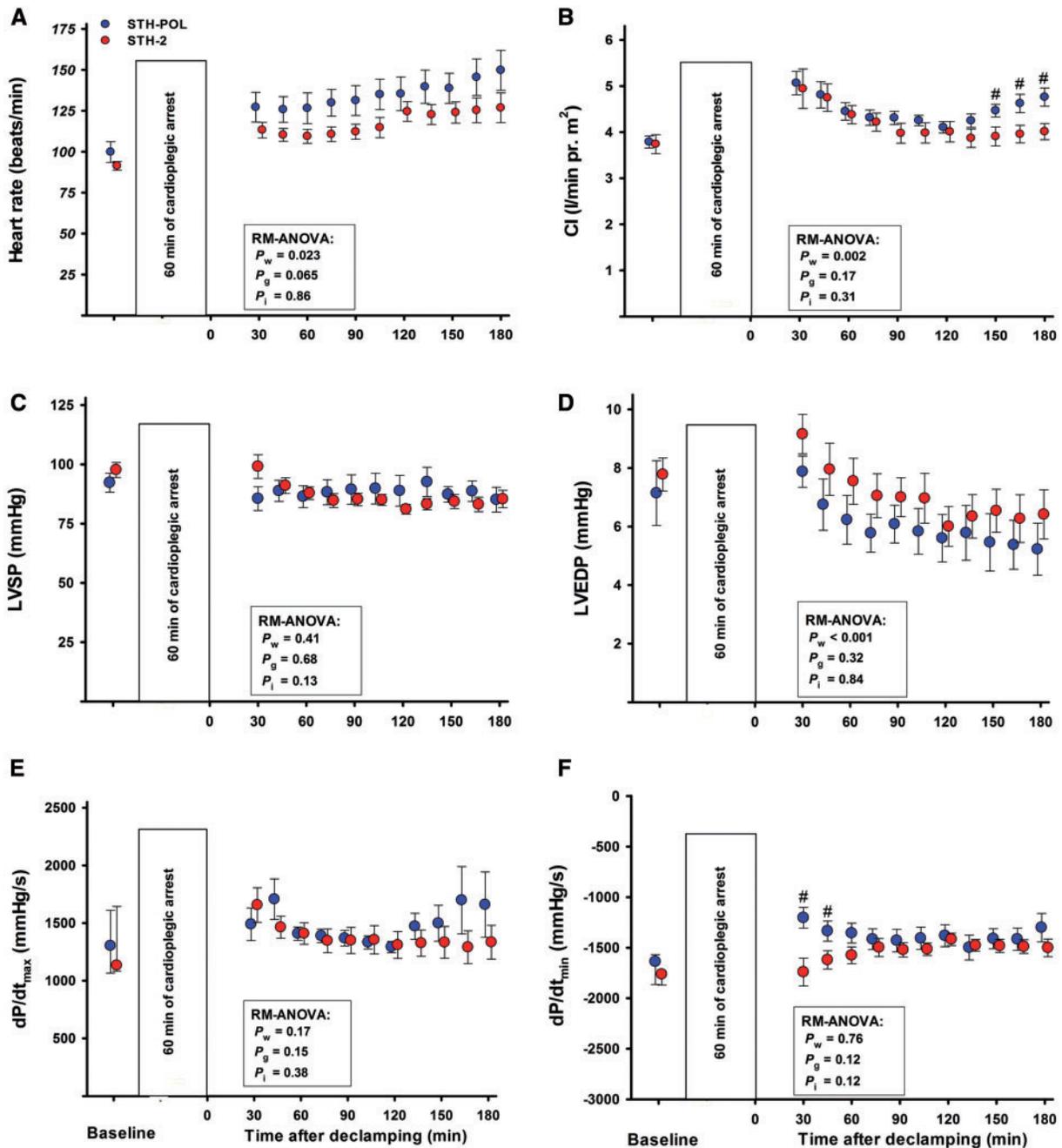


Figure 1: Left ventricular haemodynamic variables, mean (SE) or median (25%; 75%), at Baseline and after CPB and 60 min of cardiac arrest with polarizing (STH-POL; $n = 10$) and depolarizing (STH-2; $n = 10$) cardioplegia. CI: cardiac index; LVSP and LVEDP: left ventricular peak systolic- and end-diastolic pressures; dP/dt_{max} and dP/dt_{min} : peak positive and peak negative of the first derivative of left ventricular pressure; RM-ANOVA: analysis of variance for repeated measurements; P_w , P_g and P_i : P -values for within subjects, between groups and interaction from two-way RM-ANOVA, respectively; #: significantly different from STH-2 at corresponding time after declamping with Bonferroni tests.

DISCUSSION

A transient improvement in energy status with STH-POL cardioplegia was demonstrated as an increase in the myocardial tissue content of CrP after 58 min of cardioplegic arrest and at 20 min after reperfusion and weaning from CPB compared to STH-2 cardioplegia. Also, tissue ATP content was increased at 20 min after reperfusion (Fig. 2A and B).

One of the components of the STH-POL cardioplegia is adenosine, an endogenous purine nucleoside binding to the A_1

receptor. Adenosine reduces cAMP by inhibiting adenylyl cyclase and thereby acts as a K_{ATP} -channel opener. This causes outward K^+ flux and induces polarized cardiac arrest. The polarization effect is especially seen on myocardial conductive tissue such as the atrioventricular node [10]. Esmolol is a cardio-selective β_1 adrenergic receptor blocker with rapid onset by blocking the fast Na^+ - and L-type Ca^{2+} -channels initiating polarized arrest [11]. Clinically, esmolol seems to give protection from arrhythmias and is protective in a vulnerable period [12]. In clinically relevant animal studies esmolol as pre-treatment or as adjuvant to

Table 1: Baseline variables before cardiac arrest with polarizing (STH-POL) and depolarizing (STH-2) cardioplegia

Variable	STH-POL	STH-2	Statistics
HR (beats/min)	100 ± 6	91 ± 3	<i>P</i> = 0.23
LV-SP _{max} (mmHg)	92 ± 4	98 ± 3	<i>P</i> = 0.31
LV-EDP (mmHg)	7.1 ± 1.1	7.8 ± 0.7	<i>P</i> = 0.62
LV- <i>dP/dt</i> _{max} (mmHg/s)	1304 (1066; 1611)	1133 (1084; 1645)	<i>P</i> = 0.85
LV- <i>dP/dt</i> _{min} (mmHg/s)	-1638 (-1864; -1568)	-1763 (-1871; -1732)	<i>P</i> = 0.12
CI (L/min per m ²)	3.8 ± 0.1	3.7 ± 0.2	<i>P</i> = 0.85
τ (ms)	31.4 ± 1.1	31.9 ± 1.2	<i>P</i> = 0.76
RV-EDV _i (ml/m ²)	132 ± 5	132 ± 5	<i>P</i> = 0.97
RV-EF (%)	32 ± 2	34 ± 2	<i>P</i> = 0.44
MAP (mmHg)	79 ± 4	88 ± 3	<i>P</i> = 0.092
CVP _{mean} (mmHg)	7.3 ± 0.7	6.7 ± 0.7	<i>P</i> = 0.51
PAP _{mean} (mmHg)	17.8 ± 0.9	17.2 ± 0.8	<i>P</i> = 0.61
Arterial blood gases			
pH	7.50 ± 0.01	7.50 ± 0.01	<i>P</i> = 0.94
pCO ₂ (kPa)	5.42 ± 0.08	5.28 ± 0.08	<i>P</i> = 0.23
HCO ₃ ⁻ (mmol/l)	31.1 ± 0.5	30.1 ± 0.3	<i>P</i> = 0.10
BE (mmol/l)	7.3 ± 0.5	6.4 ± 0.3	<i>P</i> = 0.15
pO ₂ (kPa)	27.0 ± 0.3	27.4 ± 0.3	<i>P</i> = 0.41
Hb (g/dl)	7.7 ± 0.2	8.2 ± 0.5	<i>P</i> = 0.41
s-Na ⁺ (mmol/l)	140 ± 1	141 ± 1	<i>P</i> = 0.22
s-K ⁺ (mmol/l)	3.5 ± 0.1	3.6 ± 0.1	<i>P</i> = 0.74
s-Cl ⁻ (mmol/l)	102 (102; 103)	103 (101; 103)	<i>P</i> = 0.76

Values are mean ± SEM or median (25-percentile; 75-percentile), *n* = 10.

LV and RV: left and right ventricle; HR: heart rate; SP_{max} and EDP: peak systolic and end-diastolic pressure; *dP/dt*_{max} and *dP/dt*_{min}: peak positive and peak negative of the first derivative of left ventricular pressure; CI: cardiac index; EF: ejection fraction; EDV_i: end-diastolic volume indexed for body surface area; MAP: mean arterial pressure; CVP_{mean}: mean central venous pressure; PAP_{mean}: mean pulmonary artery pressure; *P*: *P*-values from two-sample *t*-tests or Mann-Whitney Rank Sum Tests.

Table 2: Cardiac and haemodynamic variables 60, 120 and 180 min after aortic declamping following 60 min of polarizing (STH-POL) or depolarizing (STH-2) cardioplegic arrest

Variable	60 min	120 min	180 min	RM-ANOVA statistics
RV-EDV _i (ml/m ²)				
STH-POL	116 ± 5	110 ± 4	105 ± 7	<i>P</i> _w = 0.24, <i>P</i> _g = 0.082, <i>P</i> _i = 0.83
STH-2*	128 ± 5	123 ± 7	121 ± 11	
RV-SV _i (ml/m ²)				
STH-POL	37 ± 2	32 ± 2	34 ± 1	<i>P</i> _w < 0.001, <i>P</i> _g = 0.46, <i>P</i> _i = 0.10
STH-2*	41 ± 2	35 ± 2	32 ± 2	
RV-EF (%)				
STH-POL	32 ± 2	29 ± 2	34 ± 2	<i>P</i> _w = 0.12, <i>P</i> _g = 0.32, <i>P</i> _i = 0.085
STH-2*	33 ± 2	29 ± 2	27 ± 2	
MAP (mmHg)				
STH-POL	67 ± 5	69 ± 8	63 ± 5	<i>P</i> _w = 0.49, <i>P</i> _g = 0.34, <i>P</i> _i = 0.42
STH-2	76 ± 3	69 ± 3	71 ± 4	
CVP _{mean} (mmHg)				
STH-POL	9.1 ± 0.6	9.8 ± 0.9	10.0 ± 0.8	<i>P</i> _w = 0.030, <i>P</i> _g = 0.52, <i>P</i> _i = 0.56
STH-2	8.4 ± 0.8	8.6 ± 1.1	9.7 ± 1.0	
PAP _{mean} (mmHg)				
STH-POL	25 ± 2	26 ± 2	25 ± 2	<i>P</i> _w = 0.25, <i>P</i> _g = 0.50, <i>P</i> _i = 0.74
STH-2	23 ± 2	25 ± 2	24 ± 2	

Values are mean ± SEM for 10 animals in each group (**n* = 9).

RV: right ventricle; _i: value indexed for body surface area; EDV: end-diastolic volume; SV: stroke volume; EF: ejection fraction; MAP: mean arterial pressure; CVP_{mean}: mean central venous pressure; PAP_{mean}: mean pulmonary artery pressure. *P*_w, *P*_g and *P*_i: *P*-values for within subjects, between groups and interaction from two-way RM-ANOVA, respectively.

potassium-based oxygenated blood cardioplegia improves left ventricular contractility after weaning from CPB [13, 14]. Both adenosine (*T*_{1/2}~30s) and esmolol (*T*_{1/2}~9min) have short half-lives and the elimination does not depend on hepatic or kidney

function [15]. These two arresting agents act synergistically, resulting in the desired effect at relatively low but effective concentrations, while minimizing potential adverse effects. Magnesium, included in comparable concentrations both in the STH-POL and

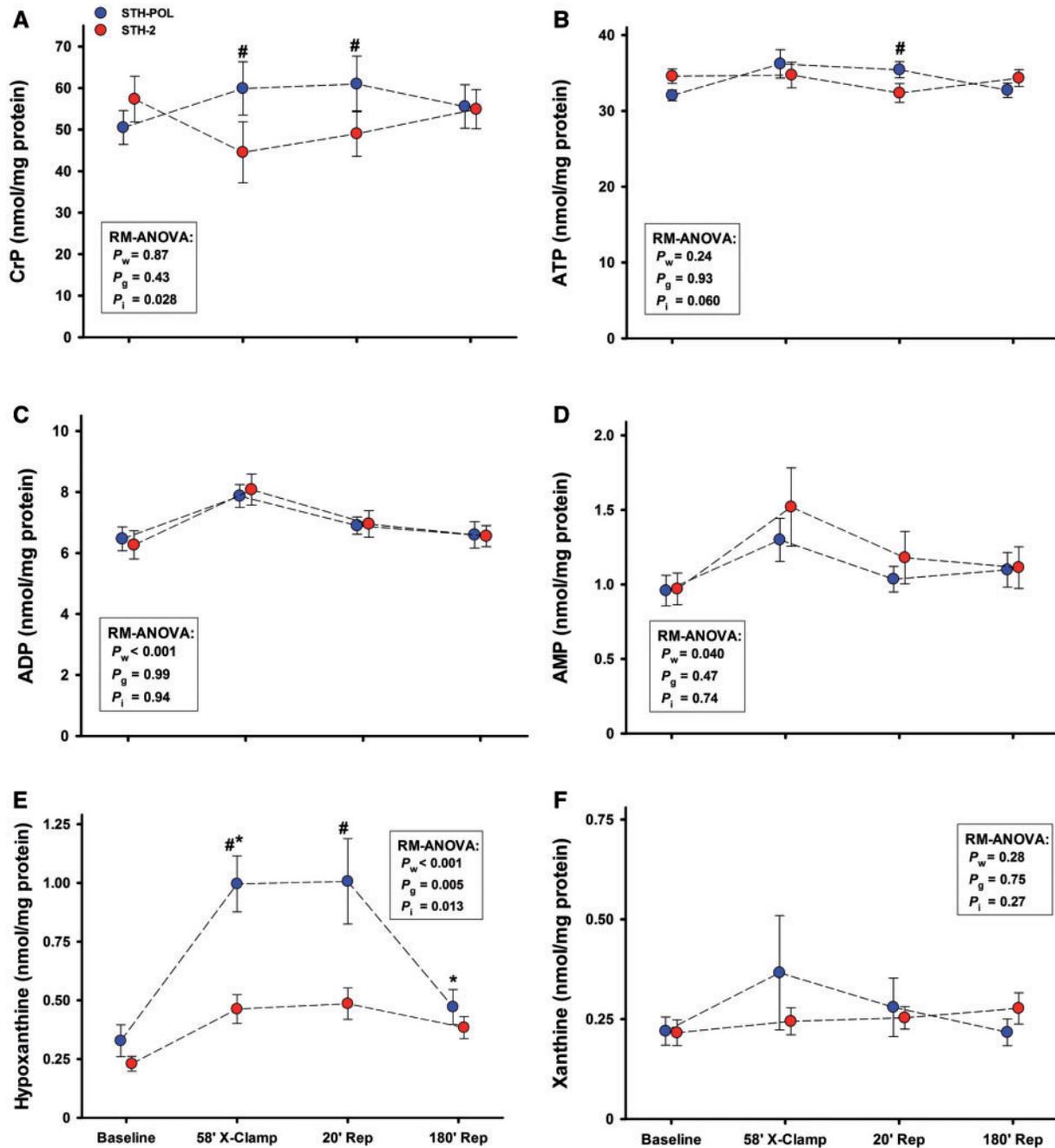


Figure 2: Myocardial tissue levels of creatine phosphate, high energy phosphates and degradation products at Baseline, after 58 min of cardiac arrest with polarizing (STH-POL) and depolarizing (STH-2) cardioplegia, and 20 and 180 min after aortic declamping and reperfusion, $n = 10$ in both groups. CrP: creatine phosphate; ATP, ADP and AMP: adenosine tri-, di- and monophosphate. P_w , P_g and P_i : P -values for within subjects, between groups and interaction from two-way RM-ANOVA, respectively; *: significantly different from the previous value in the STH-POL group; #: significantly different compared to STH-2 at the corresponding time after declamping with *post hoc* multiple contrast tests.

the STH-2 cardioplegia used in the present study suppresses intracellular Ca^{2+} overload during cardioplegia [16].

Mitochondrial creatine kinase present in the mitochondrial intermembrane space regenerates CrP from creatine imported from the cytosol and mitochondrial ADP. In the cytosol CrP serves as an energy reservoir for the rapid buffering and regeneration of ATP *in situ*, as well as for intracellular energy transport by the phosphocreatine shuttle or circuit [17, 18]. Our finding indicates a decrease in consumption of CrP during the 60 min of cardioplegic arrest and early reperfusion in the STH-POL group

(Fig. 2A). When reperfusion and contraction is re-established, the differences between groups fade with time.

Since contraction is ceased during cardioplegic arrest in both groups, the difference in CrP consumption is probably related to reduced energy consumption with polarizing cardioplegia for maintaining intracellular ion balance. For STH-2 cardioplegia intracellular Na^+ and Ca^{2+} overload occurs as a result of extracellular hyperkalaemia. The energy dependent ion channel pumps in the cellular membrane, Na^+/K^+ -ATPase, Ca^{2+} channels and the sarcoplasmic Ca^{2+} channel are active in correcting the imbalance

Table 3: Energy status in myocardial tissue at Baseline, after 58 min of cardioplegic arrest with STH-POL or STH-2 cardioplegia; and 20 and 180 min after declamping and weaning from CPB

	Baseline	58 min Cross-clamp	20 min reperfusion	180 min reperfusion	RM-ANOVA
Energy charge					
STH-POL	0.89 ± 0.01	0.88 ± 0.01	0.90 ± 0.01	0.89 ± 0.01	$P_w = 0.025, P_g = 0.75, P_i = 0.32$
STH-2	0.90 ± 0.01	0.87 ± 0.01	0.88 ± 0.01	0.90 ± 0.01	
ATP/ADP					
STH-POL	5.10 ± 0.29	4.66 ± 0.25	5.25 ± 0.32	5.11 ± 0.30	$P_w = 0.009, P_g = 0.84, P_i = 0.17$
STH-2	5.73 ± 0.35	4.48 ± 0.37	4.82 ± 0.34	5.35 ± 0.27	
CrP/ATP					
STH-POL	1.57 ± 0.11	1.68 ± 0.16	1.69 ± 0.16	1.70 ± 0.15	$P_w = 0.28, P_g = 0.36, P_i = 0.16$
STH-2	1.65 ± 0.15	1.26 ± 0.20	1.48 ± 0.13	1.60 ± 0.12	

Mean ± SEM, $n = 10$.

Energy charge (EC) calculated as: $EC = (ATP + 0.5 ADP)/(AMP + ADP + ATP)$.

ATP and ADP: adenosine tri- and diphosphate; CrP: creatine phosphate. P_w , P_g and P_i : P-values for within subjects, between groups and interaction from two-way RM-ANOVA, respectively.

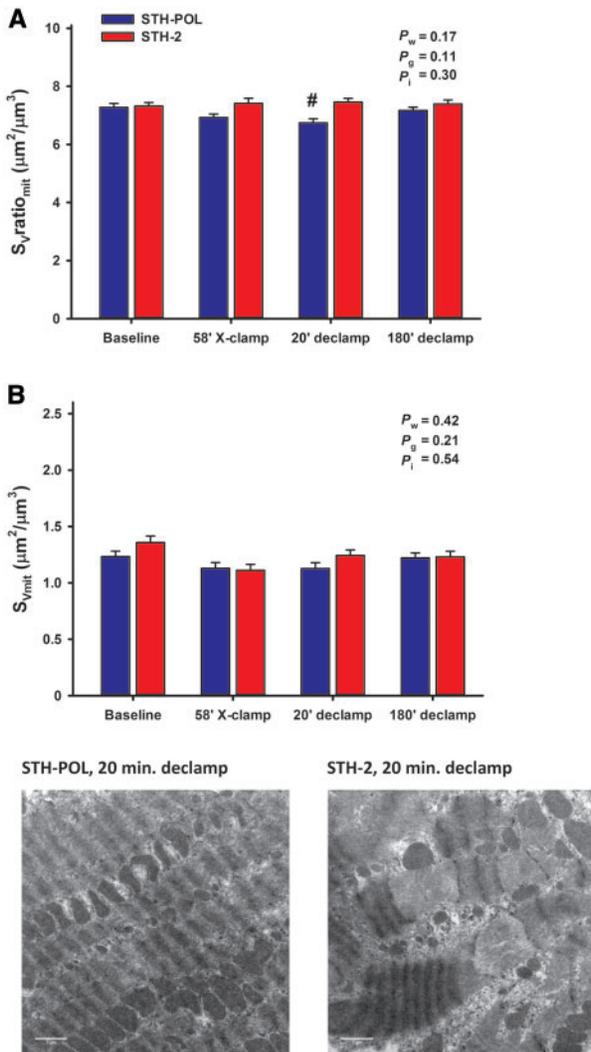


Figure 3: Myocyte mitochondrial surface to volume ratio ($S_{v\text{ratio}_{mit}}$) (A) and surface density (S_{vmit}) (B) in 10 animals in the STH-POL group and 9 animals in the STH-2 group. Bars are mean ± SEM. P_w , P_g and P_i : P-values for within subjects, between groups and interaction from two-way nested ANOVA, respectively; #: significantly different from STH-2 at 20 min after declamping. One micrograph from each group with morphometric readings close to mean values at 20 min after declamping.

in cytosolic ion composition. Since the Na^+ and Ca^{2+} overload is not prominent with polarizing cardiac arrest, these ion pumps are less active during the phase of cardioplegic arrest. As a consequence the requirement for CrP to rephosphorylate ADP to maintain ATP levels is less pronounced (Fig. 2A). In addition, CrP also stabilizes the membrane phospholipid bilayer by binding to the polar heads and decreasing membrane fluidity. Stabilizing the membrane attenuates some of the damage caused by transient ischaemia and hypoxia and helps to prevent cytoplasmic leakage [1, 4, 17].

The consumption of ATP resulted in an increase in both tissue ADP and AMP after 58 min of cardioplegic arrest in both groups. The degradation product hypoxanthine is increased both during arrest and at 20 min after declamping and reperfusion in the STH-POL compared to the STH-2 group. The surplus myocardial hypoxanthine in the STH-POL group is washed out with time during reperfusion and not oxidized to xanthine due to minimal levels of xanthine oxidase in the pig heart [19].

The decrease in surface-to-volume ratio of mitochondria at 20 min after reperfusion and declamping with STH-POL cardioplegia, indicates a temporary mitochondrial swelling (Fig. 3A). Mitochondrial swelling can be observed as early as 10 min after regional myocardial ischaemia [20]. Judged from the tissue levels of HEP, there were no signs of a more severe ischaemia in the STH-POL group than the STH-2 group. We speculate that this could be a result of transient differences in intracellular ionic composition and osmolality. Cytoplasmic volume fractions of cellular components did not differ (Table 4).

Transient differences were observed for energy metabolism and morphology at the end of the ischaemic period and at 20 min after declamping and reperfusion. Cardiac index was increased in the STH-POL compared to the STH-2 group from 150 min of reperfusion and onwards (Fig. 1B). This improved function could be interpreted as a reduction of the ischaemia/reperfusion injury, reduced myocardial stunning or alleviated adrenergic desensitization with STH-POL compared to STH-2 cardioplegia. In the present study myocardial injury was not evaluated. However, with a similar protocol in a recent study showed that STH-POL cardioplegia did not affect the release of troponin-T or myocardial caspase-3 activity as a sign of apoptosis [5]. Whether the transient differences in energy metabolism indicate a reduction of reversible myocardial

Table 4: Myocyte volume fraction (%) of mitochondria, myofibrils and cytosol with STH-POL ($n = 10$) or STH-2 ($n = 9$) cardioplegia before, after 58 min of cardioplegic arrest, and 20 and 180 min after declamping and weaning from CPB

	Baseline	58 min Cross-clamp	20 min Reperfusion	180 min Reperfusion	Nested RM-ANOVA
V_{mit} (%)					
STH-POL	20.86 ± 0.87	20.59 ± 1.02	21.25 ± 0.96	20.43 ± 0.80	$P_w = 0.94, P_g = 0.49, P_i = 0.68$
STH-2	21.30 ± 0.96	18.80 ± 0.99	20.51 ± 0.95	20.58 ± 0.94	
V_{myo} (%)					
STH-POL	62.21 ± 1.14	59.98 ± 1.29	59.45 ± 1.36	61.10 ± 1.36	$P_w = 0.47, P_g = 0.72, P_i = 0.75$
STH-2	61.53 ± 1.28	60.27 ± 1.45	61.56 ± 1.13	60.85 ± 1.13	
V_{cyt} (%)					
STH-POL	16.86 ± 0.93	19.53 ± 1.00	19.02 ± 1.09	18.45 ± 0.84	$P_w = 0.37, P_g = 0.84, P_i = 0.80$
STH-2	17.08 ± 0.80	20.91 ± 1.21	18.09 ± 0.89	18.57 ± 0.91	

Mean ± SEM, 6 randomly selected micrographs from each sample.

V_{mit} , V_{myo} , V_{cyt} : volume fraction of mitochondria, myofibrils and cytosol; P_w , P_g and P_i : P -values for within subjects, between groups and interaction from two-way nested RM-ANOVA, respectively.

Table 5: Intraclass correlation coefficient (ICC) and coefficient of variation (CV%) for inter- and intra-observer variability for morphometry data obtained from 6 electron microscopy pictures from each of 15 randomly selected biopsies

Variable	Inter-observer		Intra-observer	
	ICC	CV (%)	ICC	CV (%)
V_{mit}	0.897	6.04	0.852	5.53
V_{myo}	0.741	2.48	0.719	3.33
V_{sarc}	0.780	8.14	0.566	13.23
$S_{VratioMi}$	0.790	4.05	0.853	2.70
S_{VMi}	0.871	5.31	0.816	5.73

V_V : volume fraction; mit : mitochondria; myo : myofibrils; $sarc$: sarcoplasm; $S_{VratioMi}$: mitochondrial surface to volume ratio; S_{VMi} : mitochondrial surface density.

injury appearing as increased cardiac index after 150 min, cannot be answered by this study.

Limitations

The study is performed in young pigs with healthy hearts. Both the ischaemic time of 60 min and the 180 min of observation after declamping and weaning from CPB are relatively short. With regard to the development of myocardial stunning, the observation after weaning may have been extended. On the other hand, the stability of the experimental protocol is also of importance. The results in the present study could be directly compared with a recent and similar study focusing on left ventricular function [5]. The serial tissue biopsies obtained demanded an infrequent need for a haemostatic myocardial suture. For this reason, troponin-T was not evaluated in this study.

CONCLUSION AND IMPLICATIONS

These results demonstrate an improved energy status with polarizing oxygenated blood cardioplegia with esmolol/adenosine/

magnesium (STH-POL) compared to the standard potassium-based depolarizing blood cardioplegia (STH-2) during 60 min of cardioplegic arrest and at early reperfusion. Myocardial function was improved from 150 min after reperfusion and onwards. Comparable results should be obtained in clinical trials before implementation into a new clinical routine.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

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