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Anaesthetic Postconditioning at the Initiation of CPR Improves Myocardial and Mitochondrial Function in a Pig Model of Prolonged Untreated Ventricular Fibrillation

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CONFLICT OF INTEREST STATEMENT

None of the authors has any financial and personal relationships with other people or organisations that could inappropriately have influenced this work.

Abstract

Background—Anaesthetic postconditioning (APoC) attenuates myocardial injury following coronary ischaemia/reperfusion. We hypothesised that APoC at the initiation of cardiopulmonary resuscitation (CPR) will improve post resuscitation myocardial function along with improved mitochondrial function in a pig model of prolonged untreated ventricular fibrillation.

Methods—In 32 pigs isoflurane anaesthesia was discontinued prior to induction of ventricular fibrillation that was left untreated for 15 min. At the initiation of CPR, 15 animals were randomised to controls (CON), and 17 to APoC with 2 Vol% sevoflurane during the first 3 min CPR. Pigs were defibrillated after 4 min of CPR. After return of spontaneous circulation (ROSC), isoflurane was restarted at 0.8-1.5 Vol% in both groups. Systolic and diastolic blood pressures were measured continuously. Of the animals that achieved ROSC, 8 CON and 8 APoC animals were randomised to have their left ventricular ejection fraction (LVEF%) assessed by echocardiography at 4 hrs. Seven CON and 9 APoC were randomised to euthanasia 15 min after ROSC to isolate mitochondria from the left ventricle for bioenergetic studies.

Results—ROSC was achieved in 10/15 CON and 15/17 APoC animals. APoC improved haemodynamics during CPR and post-CPR LVEF%. Mitochondrial ATP synthesis, coupling of oxidative phosphorylation and calcium retention capacity were improved in cardiac mitochondria isolated after APoC.

Conclusions—In a porcine model of prolonged untreated cardiac arrest, APoC with inhaled sevoflurane at the initiation of CPR, is associated with preserved mitochondrial function and improved post resuscitation myocardial dysfunction.

Keywords

Cardiac Arrest; Cardiopulmonary resuscitation; Ischaemia reperfusion injury; Mitochondria; Postconditioning; Return of spontaneous circulation; Sevoflurane

INTRODUCTION

With an estimated 350,000 patients per year in the United States alone[1] and a survival rate of only 3 to 16%,[2] out-of-hospital cardiac arrest (OHCA) continues to be a significant cause of neurologic[3] and cardiac[4] morbidity and mortality. We have recently shown that ischaemic *post*conditioning at the initiation of standard[5] or sodium nitroprusside-enhanced cardiopulmonary resuscitation (CPR)[6] significantly improved neurologically intact survival following 15 min of untreated ventricular fibrillation (VF) and concomitant global ischaemia in a porcine model of cardiac arrest. CPR was augmented by the use of an active compression/decompression (ACD) device[7] and an impedance threshold device (ITD).[8] *Post*conditioning was achieved by three to four 20-sec pauses during the first 3 min of CPR. Alternatively, myocardial *post*conditioning can also be achieved by pharmacological means. Volatile anaesthetics have been shown to attenuate myocardial injury following coronary ischaemia/reperfusion (IR) in isolated hearts[9] as well as in vivo,[10] and can be administered by ventilation. In contrast, an intravenous (IV) drug would require establishing IV access and could therefore be administered only significantly *after* initiation of CPR in the OHCA setting as delaying CPR to reach a therapeutic level at the *initiation* of CPR is not feasible.

In this investigation we tested the hypotheses that the volatile anaesthetic sevoflurane given for 3 min immediately at the initiation of CPR can a) improve early post-resuscitation cardiac mitochondrial function, and b) improve post-resuscitation left ventricular function in a pig model of prolonged untreated VF.

MATERIALS AND METHODS

This study conformed to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press 2011) and was approved by the Institutional Animal Care Committee of the Minneapolis Medical Research Foundation of Hennepin County Medical Center (protocol number 11-05). All experiments were performed on isoflurane-anaesthetised female Yorkshire farm pigs weighing an average of 38.2 ± 2.1 kg.

PREPARATION

Our protocol has been described in detail.[5] After endotracheal intubation, an anaesthesia machine (Narkomed 4A, Dräger, Telford, PA, USA) was used to ventilate the animals with a tidal volume of 10 ml kg^{-1}, starting at a respiratory rate of 10-18 min^{-1} and on room air supplemented with O_2, all titrated to achieve normocapnia and an O_2 saturation ≥ 95%. Until induction of VF, general anaesthesia was maintained with inhaled isoflurane 0.8-1.5 Vol% end-tidal measured by a gas analyser (Datex-Ohmeda Capnomac; GE Healthcare, Waukesha, WI, USA) and at a fresh gas flow of 2 l min^{-1}; isoflurane was restarted after return of spontaneous circulation (ROSC) following VF and CPR. A warming blanket (Bair Hugger, Augustine Medical, Eden Prairie, MN, USA) was used to maintain body temperature at 37.5 ± 0.5°C. Arterial blood was sampled for blood gas analysis (Gem 3000, Instrumentation Laboratories, Lexington, MA, USA) at five time points: at baseline, at the end of CPR, and 5 min, 15 min and 1 hr after ROSC. Micromanometer-tipped catheters (Mikro-Tip Transducer, Millar Instruments, Houston, TX, USA) were used to continuously record central aortic (AoP) and right atrial (RAP) pressures. During CPR, coronary perfusion pressure (CPP) was calculated as the difference between AoP and RAP during diastole (spontaneously beating) or decompression (CPR). Systolic (SBP) and diastolic (DBP) blood pressure was derived from AoP. CPR compression force, rate, and depth were controlled and continuously recorded during all experiments to assure that all groups received identical CPR quality.

EXPERIMENTAL PROTOCOL

When arterial O_2 saturation on room air was ≥ 95%, and endtidal CO_2 was stable between 35 and 42 mmHg for 5 min, a direct intracardiac current was used to induce VF. Thirty-two (32) animals were used (Figure 1). Seventeen (17) animals were randomised to the APoC group and received inhaled sevoflurane for the first 3 min of CPR at an endtidal concentration of 2.0 ± 0.2 Vol% (1 minimal alveolar concentration), a clinically relevant concentration shown to significantly improve haemodynamic outcome in a rat model of cardiac arrest and resuscitation.[11] Fifteen (15) animals were randomised to the control (CON) group and did not receive any anaesthetic during CPR.

Within the APoC group, 9 of 9 randomised animals were euthanised 15 min after ROSC in order to assess mitochondrial function and reperfusion injury, and 8 animals were randomised to be kept alive for 4 hrs to echocardiographically assess left ventricular function (see below) and biomarkers of cardiac injury. Only 6 animals of the latter group survived the full 4 hrs. These 6 and the 9 animals in the mitochondrial group constituted 15 animals that achieved ROSC out of 17 in the APoC group. The same post-ROSC protocol was used for the 15 animals in the CON group; 7 animals were randomised to harvest tissue 15 min post ROSC, and 8 animals were randomised to survive up to 4 hrs. Five animals in each CON subgroup reached the time endpoints for data analysis (10 in total, Figure 1). All assignments were predetermined at the initial point of randomisation between APoC and CON groups.

In all animals, ACD CPR was performed with a pneumatically driven automatic piston (Pneumatic Compression Controller, Ambu International, Glostrup, Denmark). Uninterrupted chest compressions with a rate of 100 min^{-1}, a 50% duty cycle and a compression depth of 25% of the antero-posterior chest diameter were delivered for 4 min prior to defibrillation. Ventilation parameters remained unchanged from before induction of VF. Return of blood flow from the inferior and superior vena cava to the heart was enhanced in both groups by the use of an ITD (ResQPOD™, Advanced Circulatory Systems Inc, Roseville, MN) during CPR. If ROSC was not achieved at the first cycle, hearts were defibrillated every 2 min thereafter during CPR. Resuscitation efforts continued until ROSC or for a total of 15 min maximum. IV adrenaline was administered in a 13 μg kg^{-1} bolus at min 3 of CPR, 60 sec before the first defibrillation, and repeated every 3 min if ROSC was not achieved. Animals were ventilated with room air during CPR.

ECHOCARDIOGRAPHY

Transthoracic echocardiography (parasternal long and short axis view) was used to assess left ventricular function starting 15 min after ROSC. Left ventricular ejection fraction (LVEF%) was estimated by two independent clinical echocardiographers blinded to the treatment. For an individual animal the mean of the two ejections fractions reported by the cardiologists was used for group calculations. If a discrepancy of more than 10% was present then LVEF% was reported based on the calculation using the Simpson's method of volumetric analysis.[12]

CARDIAC INJURY MARKERS

Serum samples for biomarkers of cardiac injury (Troponin I and CK-MB) were obtained 4 hrs after ROSC and were analysed with standard human techniques in the core laboratories of the University of Minnesota Hospital.

MITOCHONDRIAL EXPERIMENTS

All mitochondrial experiments were performed in freshly isolated cardiac mitochondria. Unless otherwise stated, all necessary chemicals were obtained from Sigma, St. Louis, MO, USA.

ISOLATION OF MITOCHONDRIA

Hearts were excised 15 min after ROSC. This shorter duration post ROSC was chosen to test for functional differences in mitochondrial function without the loss of non-viable mitochondria as it would be the case with longer reperfusion times.[13] An approximately 2 g myocardial piece from the mid left anterior descending coronary artery region was immediately placed into ice-cold *isolation* buffer containing (in mM) 200 mannitol, 50 sucrose, 5 KH_2PO_4, 5 3-(nmorpholino)propanesulfonic (MOPS), 1 EGTA, and 0.1% bovine serum albumin (BSA), pH adjusted to 7.15 with KOH. The tissue was then minced into 1-mm^3 pieces and its suspension homogenised (homogeniser 60404-01, Ingenieurbüro Zipperer, Staufen, Germany) for 30 sec in the presence of 5U ml^{-1} protease (Bacillus licheniformis), followed by another 30 sec after 10-fold dilution of the protease. Mitochondria were then isolated by differential centrifugation. The suspension was first centrifuged for 10 min at 8,000 g to remove the protease. After resuspension of the pellet in 25 ml *isolation* buffer, it was centrifuged for 10 min at 750 g to remove cellular debris. The resultant supernatant with the mitochondrial fraction was centrifuged for another 10 min further at 8,000 g. The final mitochondrial pellet was resuspended in 500 μl *isolation* buffer and kept on ice. All isolation procedures were conducted at 4°C whereas all experiments were conducted at room temperature. After determination of mitochondrial protein concentration by the Bradford method,[14] mitochondria were diluted in *experimental* buffer to a final concentration of 0.5 mg protein ml^{-1}. The *experimental* buffer contained (in mM) 130 KCl, 5 K_2HPO_4, 20 MOPS, 0.001 $Na_4P_2O_7$, and 0.1% BSA (pH adjusted to 7.15 with KOH). Dilution in *experimental* buffer ensured minimal (40 μM) carry-over of EGTA from the *isolation* buffer.

MITOCHONDRIAL ATP SYNTHESIS

Rate of mitochondrial adenosine triphosphate (ATP) synthesis was determined by chemiluminescence measured in a Modulus luminometer (Turner Biosystems, Sunnyvale, CA, USA) utilising the reaction of firefly luciferase and luciferin with ATP. The solution contained *experimental* buffer, 0.2 μM diadenosine pentaphosphate, 30 μM ADP, 10 μg ml^{-1} mitochondria, 0.1 mg ml^{-1} luciferin, and 1.25 μg ml^{-1} luciferase.[15] Addition of pyruvate/malate or succinate (5 mM each) initiated the reaction that was measured for 120 sec. Defined ATP concentrations were used to obtain the standard curve.

MITOCHONDRIAL OXYGEN CONSUMPTION

Mitochondrial O_2 consumption was measured polarographically using a Clark-type O_2 electrode (Model 1302; Strathkelvin Instruments, Glasgow, Scotland) in a water-jacketed 500-μl chamber (Model MT200A; Strathkelvin Instruments), equipped with a Teflon-coated magnetic stirring bar and monitored by an O_2 meter (Model 782; Strathkelvin Instruments).[16] State 2 respiration was initiated 60 sec after sealing the chamber by adding 10 mM of the complex I substrates pyruvate and malate or a combination of the complex II substrate succinate with the complex I blocker rotenone (10 μM, dissolved in dimethyl sulfoxide). Addition of 250 μM adenosine diphosphate (ADP) at 120 sec initiated state 3 respiration, until complete phosphorylation of ADP to ATP led to state 4 respiration. Chamber O_2 concentration in μM was monitored for 60 sec after state 4 respiration was

achieved or until the O_2 concentration was 0. The respiratory control index (RCI) was calculated as the ratio of the rate of state 3 to state 4 respirations. All individual results are the average of duplicate runs.

MITOCHONDRIAL CALCIUM RETENTION AND MPTP OPENING

Mitochondria were suspended in *respiration* buffer inside a cuvette-based spectrofluorometer (LS 55, Perkin Elmer, Waltham, MA, USA) containing 10 mM of the complex I substrates pyruvate/malate or 10 mM of the complex II substrate succinate. Extra-mitochondrial (em)[Ca^{2+}] was monitored using the fluorescent probe CaGreen-5N hexapotassium salt (100 nM; Life Technologies, Grand Island, NY, USA) at excitation and emission wavelengths of 503 and 532 nm, respectively.[17] After a 1-min stabilisation period, $CaCl_2$ (25 mM) was infused at a rate of 10 μl min^{-1} to add 25 μM min^{-1} until em[Ca^{2+}] reached a steady state (equilibrium between Ca^{2+} infusion and mitochondrial Ca^{2+} uptake). With this continuous infusion of $CaCl_2$, a sudden increase in em[Ca^{2+}] is due to release of mitochondrial Ca^{2+} indicating mitochondrial permeability transition pore (mPTP) opening. The amount of $CaCl_2$ infused until mPTP opening indicated calcium retention capacity.

STATISTICAL ANALYSIS

All results are expressed as mean ± standard deviation (SD) or as percentages. The haemodynamics during CPR, blood gases and echocardiographic data up to 15 min include all surviving animals randomised to the two groups. Echocardiographic data at 1 and 4 hrs include only data from animals that were randomised to those endpoints for each group, had successful ROSC and survived to this point (Figure 1). The number (n) of animals in each group and at each time point is provided in parentheses. Unpaired student t-tests, Mann-Whitney U tests and Chi-square tests were used to calculate differences between the two experimental groups for parametric, non-parametric and categorical data, respectively. Statistical significance (*) was assumed when $P<0.05$ (two-tailed).

RESULTS

There were no significant differences between treatment groups in baseline parameters (Tables 1-3) or isoflurane concentrations before and after CPR.

CPR HAEMODYNAMICS, ROSC AND OUTCOME MEASURES

ROSC was achieved in 10 of 15 CON and 15 of 17 APoC animals (P=0.80). During CPR, DBP, SBP and CPP were improved in APoC vs. CON animals that became non-significant after ROSC (Table 1). Conversely, the adrenaline dose necessary to achieve ROSC was significantly decreased in APoC animals. The number of shocks to achieve ROSC was significantly lower in the APoC group compared to CON, *3.5 ± 2.1 vs. 6.7 ± 4.9, respectively.

ARTERIAL BLOOD GASES

Arterial blood gases showed no relevant differences between the groups (Table 2).

LEFT VENTRICULAR EJECTION FRACTION AND MYOCARDIAL INJURY MARKERS

The APoC group had a significant increase in post-ROSC LVEF% at 15 min, 1 and 4 hrs (Table 3). CK-MB and troponin levels at 4 hrs were lower in the APoC group compared to CON (*7 ± 9 and *6 ± 11 vs. 37 ± 24 and 31 ± 24 ng/ml, respectively).

MITOCHONDRIAL PARAMETERS

For both complex I and complex II substrates the rates of ATP synthesis and the RCIs as markers of coupling of oxidative phosphorylation were improved, and Ca^{2+} retention capacity was enhanced in mitochondria isolated from hearts of APoC vs. CON animals (Figure 2).

DISCUSSSION

This is the first report that APoC with the volatile anaesthetic sevoflurane, when given at the *initiation* of CPR, can improve intra-CPR haemodynamics, post-resuscitation myocardial function up to 4 hrs and preserve mitochondrial function in a preclinical porcine model of prolonged cardiac arrest.

While protection by ischaemic *pre*conditioning, i.e. periods of brief IR *before* prolonged IR first described in 1986 by Murry and colleagues,[18] requires *prior* knowledge or at least a significant probability of an ischaemic insult to follow, *post*conditioning has the advantage that it can be readily employed upon reperfusion after ischaemia has already occurred. Vinten-Johansen's group[19,20] has first described and compared ischaemic *post*conditioning with *pre*conditioning one decade ago. Since then, numerous investigations have been devoted to its further characterisation and the elucidation of specific intracellular signalling pathways.[21-24]

Studies to quantify the success of *post*conditioning in the IR-treated heart have traditionally revealed a decrease in infarct size of about 20 to 50%[19-21,25-27] while post-IR function such as contractility[21,26] or biomarker release was improved by no more than 70% compared to control.[20,21,26] Consequently, our recent results on the introduction of an ischaemic *post*conditioning protocol by limited interruptions of chest compressions during the first 3 min of CPR[5,6] were of a similar magnitude, with one important exception: neurologically intact survival after CPR, a decisive marker of resuscitation success, was significantly improved after prolonged untreated cardiac arrest,[5] indicating a considerable potential to improve cardiac and neurologic outcome and survival after a prolonged *global* ischaemic insult such as cardiac arrest by VF.

Alternatively to brief periods of IR, *pre*- and *post*conditioning can also be accomplished by pharmacological means. Many drugs have been reported to trigger *pre*- and/or *post*conditioning of the myocardium, e.g. adenosine and its receptor agonists,[28-30] bradykinin,[30,31] B-type natriuretic peptide,[32] opioids,[33] cyclosporine A,[34] noble gases,[35-39] and volatile anaesthetics.[11,40-42] It is commonly believed that not only *pre*- and *post*conditioning, but also ischaemic and pharmacological conditioning share the same common pathways,[21-24] including the reperfusion injury salvage kinase (RISK) pathway,

glycogen synthase kinase (GSK) 3β-phosphorylation and a delay or inhibition of mPTP opening.[43]

Although application of gases such as volatile anaesthetics or noble gases is not routinely performed in the setting of OHCA, it might display an attractive alternative to IV drug administration as airway management including endotracheal intubation is an integral part of CPR. While previous reports confirmed the feasibility of a syringe pump-based system to deliver volatile anaesthetics even without a dedicated anaesthesia machine[44] further studies need to focus on whether these can also be delivered reliably and safely via a bag-mask.

The concept of attenuation of IR injury by *post*conditioning necessitates the stimulus or drug to be applied immediately within the first few minutes of reperfusion.[45] This may also be the reason why some previously conducted studies were unable to find an improvement in neurological deficit score or cerebral cellular and molecular pathways when the anaesthetic was given *after* achievement of ROSC in a pig model of cardiac arrest for only 8 min.[37,46] Whether noble gases differ in this context and provide neurologic and myocardial protection when given later is the subject of ongoing research.[37,39] Certainly, due to their anaesthetic effects volatile anaesthetics require a tight seal and greater caution and need to be scavenged to avoid environmental contamination and potential danger to the emergency personnel when compared to noble gases. In contrast to IV drugs, however, distributed by circulation only, both types of gases require circulation and – in case of respiratory arrest positive pressure – ventilation. Continued sedation by volatile anaesthetics during therapeutic hypothermia extending their potential postconditioning effects to protect vital organs from further reperfusion injury is also subject of current investigations.[44]

In this study we show that APoC with sevoflurane, after prolonged untreated cardiac arrest, can significantly improve post resuscitation left ventricular function up to 4 hrs and can decrease myocardial injury based on biomarkers of injury such as troponin and CK-MB. These clinically relevant findings are observed in conjunction with significant improvements in ATP synthesis, coupling of oxidative phosphorylation and delay of mPTP opening in cardiac mitochondria isolated 15 min after ROSC. The effect of sevoflurane on mitochondrial function during early reperfusion may directly contribute to mitochondrial and thereby to tissue protection.[43]

Some limitations to our study need to be acknowledged. The use of isoflurane as a general anaesthetic before and after VF and CPR in both the APoC and CON group may have contributed to a smaller APoC effect of the sevoflurane as the CON hearts may also have received some degree of cardioprotection by preconditioning;[47,48] in the absence of a negative outcome, however, this is of negligible concern. We did not perform a dose response study in the APoC group as these are more difficult in larger vs smaller animals[11] due to the large number of subjects needed. Although our porcine model is the closest before clinical trials, a one-to-one translation into clinical practice may be hampered by species- and organ-dependent differences in sensitivity to IR.[49] Also, we have only used young adult female and healthy farm pigs whereas patients with VF cardiac arrest are typically of advanced age and have a variety of comorbidities, medications or other confounding factors interfering with cardioprotective strategies.[50] Finally, we have not evaluated the effect of

sevoflurane on neurological function and survival; ongoing studies will address this question.

CONCLUSION

In summary, we have shown that inhaled sevoflurane when administered for 3 min at the *initiation* of CPR, after 15 min of untreated cardiac arrest, significantly improved systemic haemodynamics during CPR and myocardial function after ROSC and did so by preserving mitochondrial function in pigs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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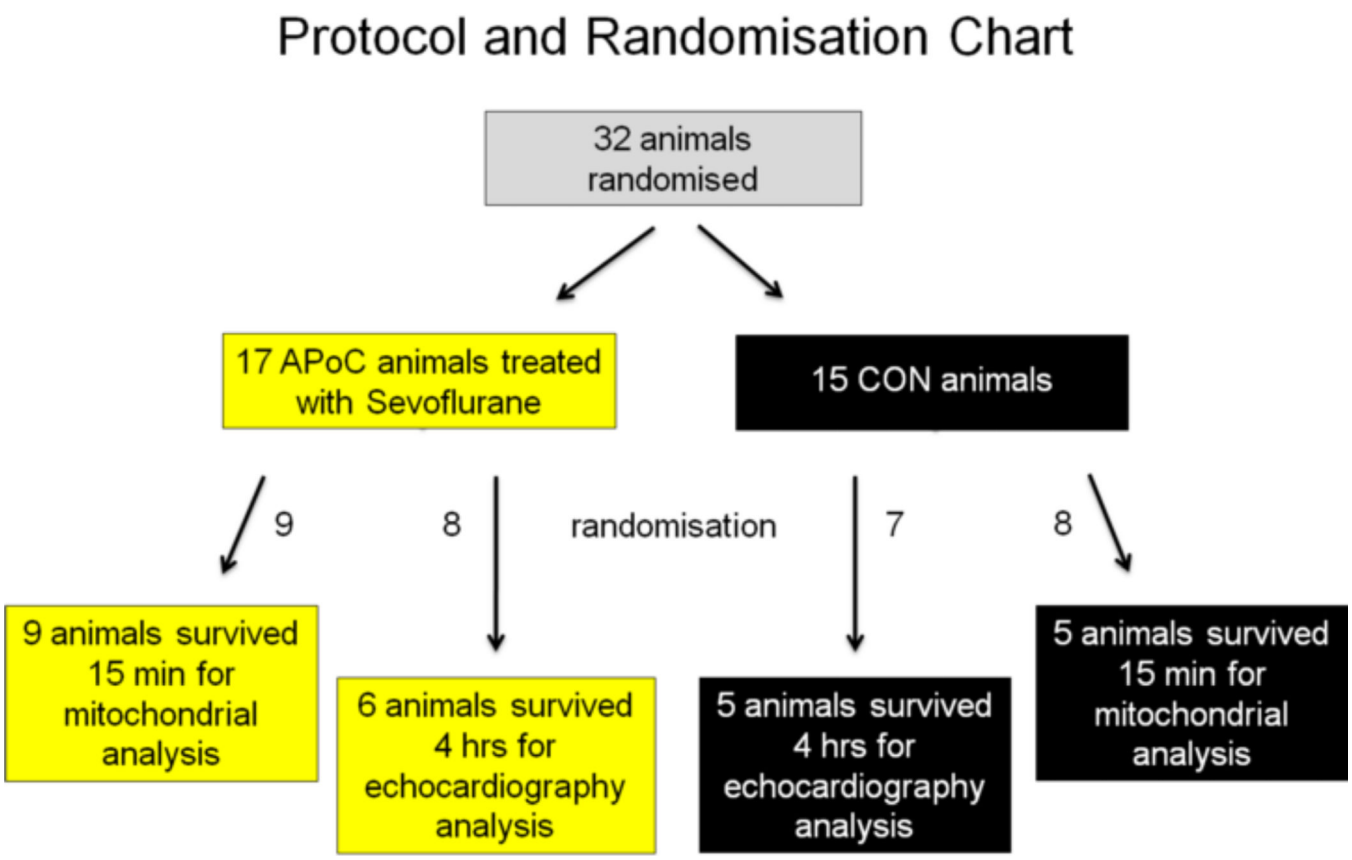

Figure 1.

Protocol and randomisation chart. Animals were initially randomised to either be treated as controls (CON) or to receive inhaled 2 Vol% sevoflurane at the initiation of CPR for 3 min with each breath (anaesthetic postconditioning, APoC). Animals were further randomised to either 15 min survival before euthanasia for mitochondrial analysis or to 4 hrs survival for left ventricular function by echocardiography and serum biomarkers of myocardial injury assessment. The number of surviving animals is stated in each box.

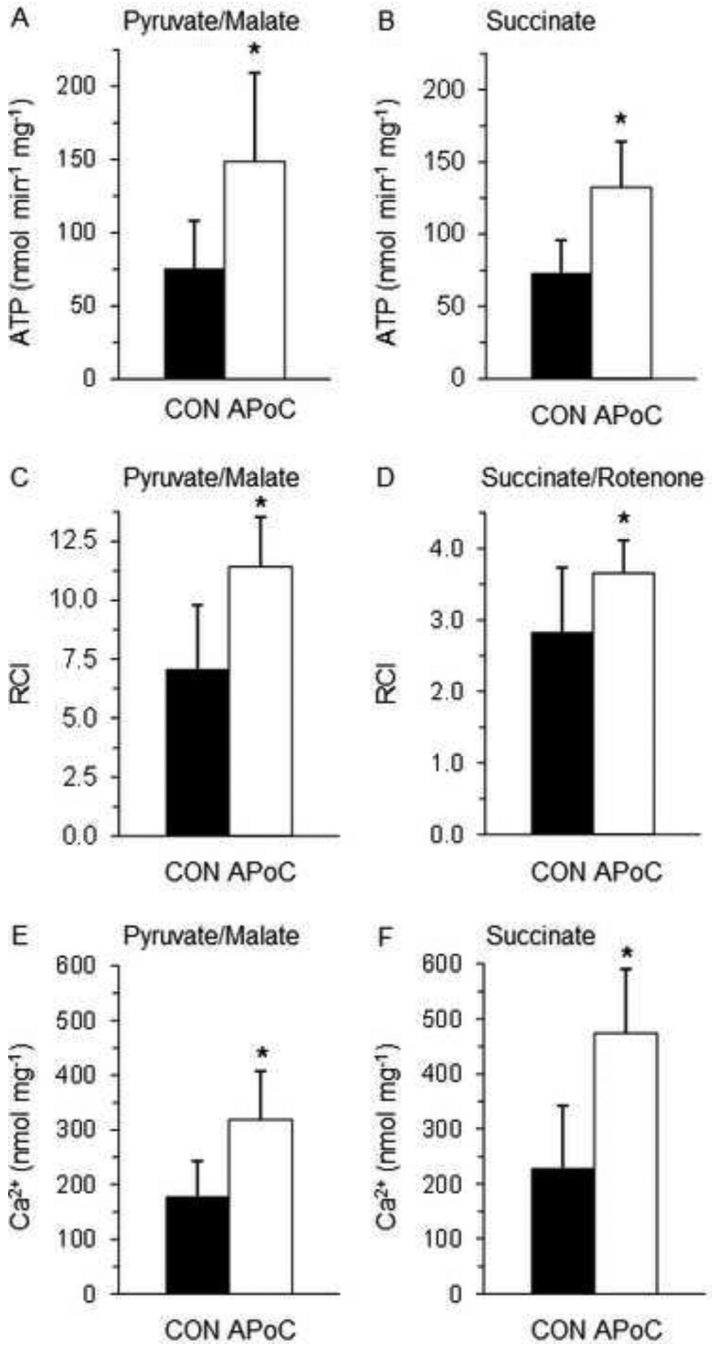

Figure 2.

Mitochondrial function studies in control (CON, n = 5, black bars) vs postconditioned hearts (APoC, n = 9, white bars). Results for mitochondrial complex I substrates pyruvate and malate are displayed on the left, results for complex II substrate succinate on the right. Panels A and B show a significantly higher rate of ATP synthesis, panels C and D better coupling of oxidative phosphorylation as measured by the respiratory control index (RCI), and panels E and F a higher Ca^{2+} retention capacity in APoC vs. CON mitochondria. All

values are mean ± SD. * Mean significantly different between groups with $P < 0.05$ (two-tailed).

Table 1

Haemodynamics and success of resuscitation.

CPR method	Parameter	Baseline	2 min CPR	4 min CPR (after adrenaline)	15 min ROSC	1 hr ROSC†	Number of shocks to initial ROSC	Total adrenaline dose (mg)	ROSC
CON	SBP	104.0±15.5 (15)	42.1±13.9 (15)	57.8±13.9 (15)	87.0±20.6 (10)	92.7±19.7 (5)	6.7±4.9 (10)	1.4±1.2 (15)	10/15
	DBP	73.1±12.4 (15)	23.0±7.4 (15)	26.6±7.7 (15)	43.7±7.6 (10)	53.4±11.2 (5)			
	RAP	3.3±2.3 (15)	3.6±1.9 (15)	3.6±1.5 (15)	7.8±3.2 (10)	6.6±1.3 (5)			
	CPP	71.6±12.8 (15)	13.9±7.0 (15)	21.7±6.2 (15)	38.1±6.6 (10)	46.8±11.2 (5)			
APoC	SBP	108.0±32.6 (17)	65.7±15.7* (17)	92.0±23.5* (17)	96.8±27.1 (15)	100.1±22.0 (6)	3.5±2.1* (15)	0.7±0.4* (17)	15/17
	DBP	80.0±16.5 (17)	27.1±8.2 (17)	44.7±9.1* (17)	59.5±18.2* (15)	54.6±7.3 (6)			
	RAP	3.5±2.1 (17)	2.2±2.1 (17)	2.9±2.9 (17)	7.7±4.6 (15)	4.4±2.2 (6)			
	CPP	77.1±17.3 (17)	25.0±7.4* (17)	41.9±8.2* (17)	52.8±18.1* (15)	50.3±14.7 (6)			

Cardiopulmonary resuscitation (CPR) was performed with either with ACD and ITD alone (CON) or with anaesthetic postconditioning (APoC) in addition. Values are shown as mean ± SD (n). Pressures are given in mmHg, flows in ml min^{-1}. SBP = systolic blood pressure, DBP = diastolic blood pressure, RAP = right atrial pressure, CPP = coronary perfusion pressure, ROSC = return of spontaneous circulation.

*Mean significantly different between groups with P<0.05 (two-tailed).

†Data 1 hr after ROSC are derived from surviving animals randomised to the 4-hr echocardiographic endpoint.

Table 2

Arterial blood gases and volatile anaesthetic concentrations.

CPR method	Parameter	Baseline	End of CPR	5 min ROSC	15 min ROSC	1 hr ROSC†
CON	pH	7.45±0.12 (15)	7.26±0.23 (15)	7.24±0.06 (10)	7.25±0.09 (10)	7.35±0.11 (5)
	pCO_2	39.7±7.4 (15)	44.7±45.3 (15)	49.5±5.7 (10)	43.5±8.2 (10)	41.0±5.1 (5)
	pO_2	129±104 (15)	91±50 (15)	134±66 (10)	132±51 (10)	102±27 (5)
	HCO_3	27.4±3.1 (15)	19.1±8.9 (15)	21.0±1.6 (10)	19.0±4.4 (10)	20.0±2.2 (5)
	SaO_2	99.4±1.2 (15)	93.7±14.7 (15)	97.5±3.8 (10)	97.3±4.1 (10)	95.0±6.7 (5)
	Isoflurane	1.1±0.2 (15)			1.0±0.2 (10)	1.0±0.2 (5)
APoC	pH	7.47±0.04 (17)	7.23±0.16 (17)	7.26±0.12 (15)	7.29±0.04 (15)	7.37±0.07 (6)
	pCO_2	37.3±3.7 (17)	51.8±20.2 (17)	42.3±7.7* (15)	38.1±6.6 (15)	39.5±4.2 (6)
	pO_2	117±91 (17)	86±33 (17)	137±112 (15)	131±70 (15)	117±54 (6)
	HCO_3	27.5±4.1 (17)	18.6±1.6 (17)	20.0±3.9 (15)	18.1±2.7 (15)	22.2±1.2 (6)
	SaO_2	99.8±0.4 (17)	93.5±5.4 (17)	97.4±6.2 (15)	97.9±5.8 (15)	98.0±3.7 (6)
	Isoflurane	1.1±0.2 (17)			1.0±0.2 (15)	1.1±0.2 (6)

Cardiopulmonary resuscitation (CPR) was performed with either with ACD and ITD alone (CON) or with anaesthetic postconditioning (APoC) in addition. Values are shown as mean ± SD (n). Arterial blood gases were measured at baseline, the end of CPR, and 5 min, 15 min and 1 hr after return of spontaneous circulation (ROSC). Partial pressures are given in mmHg. HCO_3: bicarbonate in mM; SaO_2: percent oxygen saturation. Endtidal isoflurane concentrations are given in Vol%.

*Mean significantly different between groups with $P<0.05$ (two-tailed).

†Data 1 hr after ROSC are derived from surviving animals randomised to the 4-hr echocardiographic endpoint.

Table 3

Left ventricular ejection fraction.

CPR method	Baseline	15 min ROSC	1 hr ROSC†	4 hrs ROSC†
CON	61±27 (15)	37±13 (10)	36±13 (5)	35±23 (5)
APoC	63±25 (17)	53±19* (15)	58±10* (6)	63±18* (6)

Cardiopulmonary resuscitation (CPR) was performed with either with ACD and ITD alone (CON) or with anaesthetic postconditioning (APoC) in addition. ROSC = return of spontaneous circulation. Values are shown as % mean ± SD (n).

*Mean significantly different between groups with P<0.05 (two-tailed).

†Data at 1 and 4 hrs post ROSC are derived only from surviving animals that were randomised to survive to the 4-hr echocardiographic endpoint.