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Experimental paper

Early Effects of Prolonged Cardiac Arrest and Ischemic Postconditioning during Cardiopulmonary Resuscitation on Cardiac and Brain Mitochondrial Function in Pigs

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ABSTRACT

Background: Out-of-hospital cardiac arrest (CA) is a prevalent medical crisis resulting in severe injury to the heart and brain and an overall survival of less than 10%. Mitochondrial dysfunction is predicted to be a key determinant of poor outcomes following prolonged CA. However, the onset and severity of mitochondrial dysfunction during CA and cardiopulmonary resuscitation (CPR) is not fully understood. Ischemic postconditioning (IPC), controlled pauses during the initiation of CPR, has been shown to improve cardiac function and neurologically favorable outcomes after 15 min of CA. We tested the hypothesis that mitochondrial dysfunction develops during prolonged CA and can be rescued with IPC during CPR (IPC-CPR).

Methods: A total of 63 swine were randomized to no ischemia (Naïve), 19 min of ventricular fibrillation (VF) CA without CPR (Untreated VF), or 15 min of CA with 4 min of reperfusion with either standard CPR (S-CPR) or IPC-CPR. Mitochondria were isolated from the heart and brain to quantify respiration, rate of ATP synthesis, and calcium retention capacity (CRC). Reactive oxygen species (ROS) production was quantified from fresh frozen heart and brain tissue.

Results: Compared to Naïve, Untreated VF induced cardiac and brain ROS overproduction concurrent with decreased mitochondrial respiratory coupling and CRC, as well as decreased cardiac ATP synthesis. Compared to Untreated VF, S-CPR attenuated brain ROS overproduction but had no other effect on mitochondrial function in the heart or brain. Compared to Untreated VF, IPC-CPR improved cardiac mitochondrial respiratory coupling and rate of ATP synthesis, and decreased ROS overproduction in the heart and brain.

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Conclusions: Fifteen minutes of VF CA results in diminished mitochondrial respiration, ATP synthesis, CRC, and increased ROS production in the heart and brain. IPC-CPR attenuates cardiac mitochondrial dysfunction caused by prolonged VF CA after only 4 min of reperfusion, suggesting that IPC-CPR is an effective intervention to reduce cardiac injury. However, reperfusion with both CPR methods had limited effect on mitochondrial function in the brain, emphasizing an important physiological divergence in post-arrest recovery between those two vital organs.

INTRODUCTION

Out-of-hospital cardiac arrest (CA) afflicts 395,000 people each year in the United States [1]. On average, less than 6% survive, resulting in more than 360,000 deaths per year and an enormous public health burden [2]. In the past five decades, improvements to cardiopulmonary resuscitation (CPR) and systems-based interventions have resulted in only modest improvements to outcomes following prolonged CA. Novel resuscitation strategies are necessary to further improve survival.

One such novel strategy is ischemic postconditioning (IPC), a therapy delivered upon reperfusion after prolonged ischemia to mitigate cellular injury caused by ischemia and reperfusion (IR) [3,4]. IPC is accomplished using several brief interruptions in blood flow at the onset of reperfusion. Our laboratory applied IPC during CPR in a porcine model of prolonged whole-body ischemia during ventricular fibrillation (VF) CA. Pauses in chest compressions during the first 2 min of CPR improved left ventricular ejection fraction (LVEF) following return of spontaneous circulation (ROSC) and increased neurologically favorable survival after 48 h [5–7]. These data demonstrate that the method of initial reperfusion with CPR has an impact on the extent of injury after prolonged cardiac arrest.

Mitochondria are implicated in the pathophysiology of IR injury and also provide a nexus for integrating the protective molecular pathways activated by IPC [8–10]. Therefore, we sought to investigate the effect that VF, CPR, and IPC have on mitochondrial function in the heart and brain. We hypothesized that mitochondrial function: 1) is significantly depressed after prolonged cardiac arrest, and 2) can be improved with IPC at the initiation of CPR (IPC-CPR). Mitochondrial responses to ischemia, CPR, and IPC were characterized without attempting defibrillations to eliminate confounders such as success and timing of ROSC, refractory VF, number of defibrillations, antiarrhythmics, and vasopressor support. Understanding the early physiological consequences of the key components of cardiac arrest and resuscitation will have significant impact on the direction and refinement of future therapies.

METHODS

All studies were performed with approval from the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation and the University of Minnesota in accordance with the National Research Council's Guidelines for the Care and Use of Laboratory Animals [11]. Animal preparation has been described previously [6]. See Supplement for full description of methods.

Experimental Protocols

Following surgical preparation and baseline measurements, animals were randomized to one of four groups (Naïve, Untreated VF, S-CPR, and IPC-CPR) and further to either heart or brain isolation (Fig. 1). Twelve animals received no ischemia (Naïve, heart isolation [$n = 7$], brain isolation [$n = 5$]). In the three ischemic groups, VF was induced via a pacing wire positioned in the right ventricle. During VF, ventilation and temperature management were discontinued. Mitochondria were isolated 19 min after initiation of VF in all three ischemic groups. To establish the effect of prolonged untreated VF CA, 13 animals were randomized to receive 19 min of VF CA without CPR (Untreated VF, heart [$n = 8$], brain [$n = 5$]). To establish the effect of reperfusion by type of CPR on mitochondrial function, 38 animals were randomized to receive 15 min of untreated VF arrest followed by either 4 min of standard CPR (S-CPR, heart [$n = 11$], brain [$n = 8$]) or 4 min of IPC-CPR (heart [$n = 11$], brain [$n = 8$]). Epinephrine (0.125 μg/kg) was administered after the third minute of CPR. In the IPC-CPR group, IPC was administered via three cycles of 20 s compressions/ventilations followed by 20 s pause in compressions/ventilations during the first 2 min of CPR. Both CPR groups received asynchronous ventilations at 10 breaths/min and 10 ml/kg, and 100 chest compressions/min with a target depth of 20% AP diameter and 50% duty cycle. Compressions were performed with an automated, custom-built, CPR piston device (Caztek Engineering, St. Paul, MN). Coronary perfusion pressure (CPP), the gradient between aortic and right atrial blood pressures during chest decompression, was calculated as an approximation of coronary blood flow and a measure of CPR quality. At the end of the fourth minute of CPR, tissue was sampled for mitochondria isolation. No defibrillations were attempted.

At the conclusion of the experimental protocol, mitochondria were isolated at 4 °C via differential centrifugation from the left ventricle of the heart and brain as described previously [12,13]. State 3 (S3) and State 4 (S4) respiration, the rate of ATP synthesis, and calcium retention capacity (CRC) were quantified as previously described [12]. The respiratory control index (RCI) was calculated as the ratio of S3 to S4 respiration. Production of reactive oxygen species (ROS) was assessed with electron spin resonance (ESR) fresh frozen heart and brain tissue.

Statistics

Values are expressed as mean ± standard error of the mean. ANOVA with Newman–Keuls posthoc test was used for comparisons between treatments. Unpaired two-tailed t-tests were used to compare hemodynamics during CPR between S-CPR and IPC-CPR. The null-hypothesis was rejected for $p < 0.05$.

RESULTS

Baseline hemodynamic parameters did not differ between Naïve, Untreated VF, S-CPR, or IPC-CPR groups (Table 1). As expected, pauses in chest compressions during IPC-CPR resulted in a lower average CPP during each pause compared to S-CPR (Fig. 2a). However, IPC-CPR resulted in a higher CPP during the fourth minute of CPR after administration of epinephrine. Average CPP during CPR did not differ between S-CPR and IPC-CPR (Fig. 2b).