

Video Article

A Rat Model of Ventricular Fibrillation and Resuscitation by Conventional Closed-chest Technique

Lorissa Lamoureux¹, Jeejabai Radhakrishnan¹, Raúl J. Gazmuri¹

¹Resuscitation Institute, Rosalind Franklin University of Medicine and Science

Correspondence to: Raúl J. Gazmuri at raul.gazmuri@rosalindfranklin.edu

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Abstract

A rat model of electrically-induced ventricular fibrillation followed by cardiac resuscitation using a closed chest technique that incorporates the basic components of cardiopulmonary resuscitation in humans is herein described. The model was developed in 1988 and has been used in approximately 70 peer-reviewed publications examining a myriad of resuscitation aspects including its physiology and pathophysiology, determinants of resuscitability, pharmacologic interventions, and even the effects of cell therapies. The model featured in this presentation includes: (1) vascular catheterization to measure aortic and right atrial pressures, to measure cardiac output by thermodilution, and to electrically induce ventricular fibrillation; and (2) tracheal intubation for positive pressure ventilation with oxygen enriched gas and assessment of the end-tidal CO₂. A typical sequence of intervention entails: (1) electrical induction of ventricular fibrillation, (2) chest compression using a mechanical piston device concomitantly with positive pressure ventilation delivering oxygen-enriched gas, (3) electrical shocks to terminate ventricular fibrillation and reestablish cardiac activity, (4) assessment of post-resuscitation hemodynamic and metabolic function, and (5) assessment of survival and recovery of organ function. A robust inventory of measurements is available that includes – but is not limited to – hemodynamic, metabolic, and tissue measurements. The model has been highly effective in developing new resuscitation concepts and examining novel therapeutic interventions before their testing in larger and translationally more relevant animal models of cardiac arrest and resuscitation.

Video Link

The video component of this article can be found at <https://www.jove.com/video/52413/>

Introduction

Close to 360,000 individuals in the United States¹ and many more worldwide² suffer an episode of sudden cardiac arrest every year. Attempts to restore life require not only that cardiac activity be reestablished but that damage to vital organs be prevented, minimized, or reversed. Current cardiopulmonary resuscitation techniques yield an initial resuscitation rate of approximately 30%; however, survival to hospital discharge is only 5%¹. Myocardial dysfunction, neurological dysfunction, systemic inflammation, intercurrent illnesses, or a combination thereof occurring post-resuscitation account for the large proportion of patients who die in spite of initial return of circulation. Thus, greater understanding of the underlying pathophysiology and novel resuscitation approaches are urgently needed to increase the rate of initial resuscitation and subsequent survival with intact organ function.

Animal models of cardiac arrest play a critical role in the development of new resuscitation therapies by providing insights on the pathophysiology of cardiac arrest and resuscitation and offering practical means to conceptualize and test new interventions before they can be tested in humans³. The rat model of closed chest cardiopulmonary resuscitation (CPR) described here has played an important role. The model was developed in 1988 by Irene von Planta – a research fellow at the time – and her collaborators⁴ in the laboratory of late Professor Max Harry Weil M.D., Ph.D. at the University of Health Sciences (renamed Rosalind Franklin University of Medicine and Science in 2004) and has been extensively used in the field of resuscitation predominantly by fellows of Professor Weil and their trainees.

The model simulates an episode of sudden cardiac arrest with resuscitation attempted by conventional CPR techniques and thus includes induction of ventricular fibrillation (VF) by delivering an electrical current to the right ventricular endocardium and provision of closed chest CPR by a pneumatically driven piston device while concomitantly delivering positive pressure ventilation with oxygen-enriched gas. Termination of VF is accomplished by transthoracic delivery of electrical shocks. The rat model strikes a balance between models developed in large animals (e.g., swine) and models developed in smaller animals (e.g., mice) allowing exploration of new research concepts in a well-standardized, reproducible, and efficient manner with access to a robust inventory of pertinent measurements. The model is particularly useful in early stages of research to explore new concepts and examine the effects of confounders before conducting studies in larger animal models that are more costly, but of greater translational impact.

A Medline search for all peer-reviewed articles reporting a similar rat model having VF as the mechanism of cardiac arrest and some form of closed chest resuscitation revealed a total of 69 additional original studies using the model since it was first published in 1988⁴. The research

areas include pathophysiological aspects of resuscitation⁵⁻¹⁷, factors influencing outcomes¹⁸⁻³⁰, the role of pharmacological interventions examining vasopressor agents³¹⁻⁴³, buffer agents⁴⁴, inotropic agents⁴⁵, agents aimed at myocardial or cerebral protection⁴⁶⁻⁷⁰, and also the effects of mesenchymal stem cells⁷¹⁻⁷³.

The model and protocol described in this article is currently being used at the Resuscitation Institute. Yet, there are multiple opportunities to "customize" the model based on the capabilities available to individual investigators and the goals of the studies.

Protocol

NOTE: The protocol was approved by the Institutional Animal Care and Use Committee at Rosalind Franklin University of Medicine and Science. All procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Research Council.

1. Experimental Setup and Anesthesia

1. Perform calibrations of the various signals to be captured using a data acquisition system (pressures, temperature, piston displacement, electrocardiogram [ECG], capnography, etc.).
2. Sterilize instruments and catheters (e.g., in an autoclave for instruments and ethylene oxide sterilizer for catheters) and operate gowned and wearing a mask, cap, and sterile gloves if the experiment involves survival surgery. Clean surgical instruments and catheters but there is no need to be sterile for non-survival surgery.
3. Prepare the catheters described below and depicted in **Figure 1** for a rat weighing between 0.45 kg and 0.55 kg.
 1. Mark a 2F T-type thermocouple catheter, size 0.6 mm OD (2F), at 3, 5, and 8 cm from the tip with permanent marker, for advancement into the thoracic aorta. Use this catheter to measure temperature and cardiac output.
 2. Cut polyethylene tubing, size 0.46 mm ID and 0.91 mm OD (PE25) $\approx$ 25 cm in length, one for advancement into the thoracic aortic and another for advancement into the right atrium.
 3. Cut the end of each PE25 catheter tip to be inserted into the vessel at a 90° angle.
NOTE: Beveled tips at 45° angle may cause vessel perforation when using PE tubing. However, the beveled tip can be trimmed down with sandpaper to reduce its sharpness.
 4. Attach a 26 gauge female luer stub adaptor to the proximal end of each PE25 catheter.
 5. Mark the aortic catheter at 3, 5, and 8 cm and the right atrial catheter at 3, 5, 8, 10 and 12 cm from the tip. Use the aortic catheter to measure aortic pressure and for blood sampling. Use the right atrial catheter to measure right atrial pressure.
 6. Attach each luer stub adaptor to a pressure transducer fitted with a 3-way stopcock.
 7. Cut the tip of a 3F polyurethane pediatric venous catheter, size 0.6 mm ID and 1.0 mm OD (3F), at a 45° angle for advancement into the right atrium.
 8. Mark the 3F external jugular catheter at 4 cm from the tip. Use this catheter to advance a guide wire into the right ventricle for electrical induction of VF with the subsequent option to use it for drug delivery and blood sampling. Attach a 3-way stopcock to the catheter.
NOTE: Marks made on the catheters are for the surgeon's guidance as the catheters are advanced. The mark at 3 cm on the catheters advanced through the femoral vessels alerts the surgeon of an area of potential resistance resulting from the vessels beginning to curve up towards the thoracic region. The 8 cm marks on the aortic catheter and thermocouple catheter indicate the tip is in the descending thoracic aorta. The 12 cm mark on the right atrial catheter indicates the tip is in the right atrium. Interim marks are guides as the catheters are advanced. The 4 cm mark on the right external jugular catheter indicates the tip is in the right atrium.
 9. Prime each catheter with saline containing 10 IU/ml of heparin (to ensure their patency) and turn the corresponding stopcocks to the closed position.
 10. Cut a 5F fluorinated ethylene propylene cannula, size 1.1 mm ID and 1.6 mm OD (5F) mounted on a stylette, to be $\approx$ 8 cm in length creating a blunted tip. Use this cannula for advancement into the trachea placing its tip $\approx$ 2 cm from the carina for positive pressure ventilation during and after cardiac resuscitation.
NOTE: The metal stylette of the cannula needs to be bent at a 145° angle $\approx$ 3 cm from the tip to aid in advancement into the trachea.
4. Prepare the rat for surgical instrumentation.
 1. Anesthetize the rat by intraperitoneal injection of sodium pentobarbital (45 mg/kg). If required, give additional doses (10 mg/kg) intravenously every 30 minutes (after establishing vascular access) to maintain a surgical plane of anesthesia.
NOTE: Most studies have used male retired breeder Sprague-Dawley rats.
 2. Clip the hair from the surgical areas and areas where electrical shocks will be delivered; which include the dorsal thoracic area, left and right groin, neck, and anterior surface of the thorax.
 3. Administer 0.02 mg/kg (1 ml/kg) buprenorphine subcutaneously for analgesia.
 4. Fix the rat in a supine position on a surgical board by taping the front and hind limbs at a 45° angle from the midline.
 5. Scrub incision areas with betadine scrub followed by 70% ethanol 3 times.
 6. Apply a thin film of antibacterial ophthalmic ointment to the corneas.
 7. Insert a rectal thermistor $\approx$ 4 cm into the rectum and secure the thermistor to the surgical board.
 8. Maintain core body temperature between 36.5 °C and 37.5 °C using an incandescent heating lamp throughout the experiment.
 9. Place ECG needles subcutaneously on the right upper limb, left upper limb, and the right hind limb, and record the ECG throughout the experiment.

2. Vascular Cannulations

2.1) Left femoral artery for advancing the T-type thermocouple catheter into the descending thoracic aorta

1. Make a 2 cm incision on the left inguinal area at a 90° angle relative to its groove.

2. Expose the femoral vessels and nerve by blunt dissection of the surrounding connective tissue using a pair of hemostats.
3. Expose the vascular sheath around the vessels using a curved micro dissection forceps.
NOTE: Avoid puncturing either vessel or the nerve.
4. Travel with micro dissection forceps underneath the femoral artery, vein, and nerve and support them at a 90° angle relative to the vessels. With both vessels and the nerve supported, begin separation of the artery from the nerve and vein by using another pair of curved micro dissection forceps.
NOTE: Separation is done from underneath and parallel to the vessels to minimize risk of injury to the vessels and nerve.
5. Reposition the supporting forceps; releasing the nerve to support only the vein and artery.
6. Thread a forceps between the artery and the vein and separate them to a length of ≈ 1 cm.
7. Release the isolated vein from the supporting forceps gently, and remain supporting the artery only.
8. Insert two silk 3-0 braided non-absorbable ligatures and position one distally and one proximal ≈ 1 cm apart.
9. Tighten firmly the distal ligature while the artery is still supported using a surgeon's knot followed by two single knots. Tighten the proximal ligature with a loose surgeon's knot.
10. Make a small incision on the vessel using a pair of micro dissection scissors near the distal ligature at a 60° angle relative to the vessel cutting approximately $\frac{1}{4}$ of its the cross sectional area.
NOTE: A small drop of blood emerging from the cut signals the lumen was reached.
11. Drip heparinized saline onto the vessel to allow for smooth insertion of the catheter.
NOTE: One to two drops of 1% lidocaine solution can also be used to prevent vessel spasm.
12. Insert a 22 gauge needle – whose tip has been custom bent at a 70° angle and blunted using sandpaper (*i.e.*, introducer) – into the vessel opening while gently pulling the distal ligature with the hemostats to stabilize the vessel.
13. Lift the introducer gently to expose the lumen and guide the T-type thermocouple catheter under the introducer, removing it once the catheter tip has been inserted.
14. Hold the catheter in place with one hand while accommodating the other hand in a comfortable position to advance the catheter.
15. Close the supporting forceps and move them distally as the catheter is advanced.
NOTE: If any resistance is met while advancing the catheter; stop, pull back and insert at an alternative angle.
16. Advance the catheter until the 8 cm mark to position its tip into the descending thoracic aorta.
17. Secure the catheter to the vessel by tightening the proximal ligature and adding two additional single knots.
NOTE: Secure knots tight enough to prevent bleeding around the catheter and inadvertent displacement; yet, loose enough to enable back and forth movement if required for repositioning.
18. Remove the forceps and the hemostats gently.

2.2) Left femoral vein for advancing the PE25 catheter into the right atrium

1. Lift the femoral artery already cannulated with the T-type thermocouple catheter by gently pulling up on the ligature and exposing the adjacent femoral vein.
2. Travel under the vein using forceps and open them to prop up the vein.
3. Follow steps 2.1.8 through 2.1.18 but advancing the PE25 catheter (instead of the T-type thermocouple) to the 12 cm mark to position its tip near the right atrium.
4. Verify blood can be withdrawn through the catheter to confirm its intraluminal unobstructed position and flush the catheter with 0.2 ml of heparinized saline.
5. Close the surgical incision with a single surgeon's knot.

2.3) Right femoral artery for advancing the PE25 catheter into the descending thoracic aorta

1. Follow steps 2.1.1 through 2.1.18 but advancing the PE25 catheter to the 8 cm mark to position its tip into the descending thoracic aorta.
2. Repeat steps 2.2.4 and 2.2.5.

2.4) Right external jugular vein for advancing the 3F polyurethane pediatric venous catheter into the right atrium

1. Make a 1.5 cm long incision starting at the base of the neck, 1 cm to the right of trachea, ending just below the thyroid.
NOTE: Avoid injuring or exposing the thyroid gland.
2. Gently dissect the surrounding connective tissue using a pair of hemostats to expose the external jugular vein.
3. Travel under the vein using forceps and open them to prop up the vein.
4. Repeat steps 2.1.8 through 2.1.18 for vein catheterization, but advancing the 3F catheter to the 4 cm mark positioning its tip in the right atrium.
5. Repeat step 2.2.4.
6. Cap the catheter with the 3-way stopcock and turn it to the closed position.

3. Tracheal Intubation

3.1) Tracheal exposure

1. Expand the previously performed neck incision toward the midline using hemostats.
2. Dissect with hemostats and forceps using blunt technique the sternohyoid, sternothyroid, and mastoid part of the cleidocephalic muscles to expose the trachea and hold it exposed using a tissue spreader.

3.2) Tracheal intubation

1. Pull the tongue out to stretch the airway. Advance the 5F catheter (*i.e.*, tracheal cannula) mounted on the stylette. Firmly hold the cannula while advancing with the tip pointing upward and advance seeking to enter the upper airway, vocal cords, and trachea.

2. Trans-visualize the tracheal cannula as it advances for guidance into proper position.
3. Remove the stylette from the cannula and attach an infrared CO₂ analyzer adaptor to the distal end of the cannula.
4. Confirm successful tracheal intubation by recognizing the characteristic capnographic waveform; *i.e.*, airway CO₂ increasing during expiration and decreasing during inspiration.

4. Confirmation of Baseline Stability

1. Complete the surgical instrumentation and connection of the various catheters, cannulas, and ECG leads *via* their corresponding transducers and signal conditioners to a data acquisition system, and confirm hemodynamic stability based on cardiac output and blood pressure measurements and metabolic stability (advisable) by measuring blood gases and lactate levels.
NOTE: Cardiac output is measured by computer analysis of the thermodilution curve recorded in the descending thoracic aorta through the thermocouple after 200 μ l bolus injection of 0.9% NaCl at room temperature into the right atrium.
2. Define the specific baseline reference values for the various parameters of interest; which may vary contingent on the rat strain, gender, and weight. Baseline and post-resuscitation reference values from a representative experiment using the rat model herein described are listed on Table 1.

5. Experimental Protocol

5.1) Induction of ventricular fibrillation (VF)

1. Insert a needle subcutaneously in the rat's abdominal wall connected to the negative pole of a 60 Hz, alternating current (AC) generator (0 to 12 mA). Avoid advancing the needle beyond the subcutaneous tissue into the abdominal cavity to avoid inadvertent injury to internal organs.
2. Attach one end of a precurved 0.38 mm OD and 40 cm long guide wire (via a wire connector) to the positive pole of the AC generator. Ensure that the polarity is not reversed; otherwise VF may not be induced.
3. Remove the 3-way stopcock from the 3F polyurethane catheter inserted in the right external jugular vein and advance the softer tip of the guide wire approximately 7 cm seeking to enter the right ventricle while monitoring the ECG and the aortic pressure.
NOTE: Correct placement of the guide wire will be suggested by ectopic ventricular beats observed in the ECG and aortic pressure.
4. Turn on the 60 Hz AC generator and gradually increase the current while monitoring the aortic pressure.
NOTE: A 2.0 mA current is typically sufficient to induce VF but it varies contingent on location of the guide wire relative to the right ventricle. Minor adjustments to the tip location may be required to induce VF at lower current levels.
5. Confirm induction of VF by documenting (1) cessation of aortic pulsations and exponential decay of the aortic pressure to $\approx$ 20 mm Hg within $\approx$ 5 seconds and (2) appearance of unorganized electrical activity in the ECG, as shown in Figure 2.
6. Maintain the current uninterruptedly for 3 minutes reducing the intensity after the first minute to approximately half the level required to induce VF.
7. Turn the current off after 3 min and document that VF continues without the need to apply current.
NOTE: Small hearts defibrillate spontaneously given a short circuit length whereby the leading edge of the fibrillatory front reaches its trailing end in refractory period precluding reentry. Only after a period of myocardial ischemia; *i.e.*, 3 min, enough to slow conduction to allow reentry is that VF becomes self-sustained, as shown in Figure 2.
8. Remove the guide wire, re-cap the jugular catheter with the 3-way stopcock, remove the ground needle, and allow VF to continue spontaneously for the desired duration of the protocol before starting resuscitation interventions (*i.e.*, 4 to 15 min based on published studies).

5.2) Chest compressions and positive pressure ventilation

NOTE: The chest compressor featured in this publication is a custom-made pneumatically driven and electronically controlled piston device. The ventilator is a commercially available device.

1. Use the time of untreated VF for the actions described below; although they can be performed before inducing VF.
2. Mark the chest at 2.8 cm and 4.2 cm from the base of the xiphoid process. The optimal area for initiating chest compressions is typically found between these two marks.
3. Apply conductive gel to a defibrillation paddle and slide it underneath the rat's chest, securing the paddle to the surgical board.
4. Position the piston of the chest compressor between the two chest marks slightly touching the chest.
5. Set the compressor to deliver 200 compressions per minute and set the initial piston displacement to 0 mm.
NOTE: The compression rate is appropriate for a small animal with a spontaneous heart rate of 350 min⁻¹ but it can be varied as the optimal compression rate for the rat model has not been defined.
6. Set the ventilator at 25 min⁻¹ delivering a tidal volume of 6 ml/kg and a fraction of inspired oxygen (FiO₂) of 1.0 unsynchronized to chest compression.
7. Attach the ventilator tubing (ending in a Y-adaptor connecting the inspiratory and expiratory limbs) to the tracheal cannula leaving interposed the infrared CO₂ analyzer adaptor.
8. Turn on the ventilator and start chest compression by gradually increasing the compression depth from 0 mm to 10 mm during the first minute. Move slightly the piston sideways and rostrocaudal seeking to find a position that yields the highest aortic diastolic pressure (*i.e.*, pressure between compressions) for a given compression depth.
NOTE: The gradual increase in compression depth is unique to the Resuscitation Institute; most investigators start with the maximal compression depth.
9. Continue increasing the compression depth during the second minute until a target aortic diastolic pressure is achieved.
NOTE: A target aortic diastolic pressure of 24 mm Hg or higher yields a coronary perfusion pressure of 20 mm Hg or higher after subtracting the right atrial diastolic pressure; corresponding to the resuscitability threshold for this rat model⁴. The target aortic diastolic pressure – which may exceed the resuscitability threshold – is to be decided by the investigator based on the study objective. Yet, it is not advisable to exceed a compression depth of 17 mm to avoid injury to the chest wall and intrathoracic organs.
10. Maintain chest compressions for the desired duration before attempting defibrillation.

NOTE: Six minutes of chest compression seems to be the minimum required to create myocardial conditions favorable for successful defibrillation²⁶. However, with increasing duration, the hemodynamic efficacy of chest compression declines and most studies use a duration ranging from 6 to 10 min.

5.3) Defibrillation

1. Use a commercially available biphasic waveform defibrillator with capability for internal defibrillation with a starting delivered energy of 5 J, equipped with paddles customized to the rat.
2. Apply conductive gel to the defibrillation paddle.
3. Charge the defibrillator immediately before completing the predetermined duration of chest compressions.
4. Interrupt chest compression and verify the heart remains in VF examining the ECG.
5. Deliver up to two electrical shocks of 5 J each across the chest wall 5 seconds apart if VF is present and observe for return of an electrically organized ECG with aortic pulses and a mean aortic pressure ≥ 25 mm Hg.
6. Resume chest compressions for another 30 seconds or 60 seconds (contingent on the specific protocol) if the mean aortic pressure is < 25 mm Hg regardless of the electrical rhythm.
7. Repeat the steps from 5.3.4 to 5.3.6 for up to 5 times contingent on the specific protocol but escalating the defibrillation energy to 7 J if the initial 5 J shocks fail to terminate VF. Figure 3 depicts the defibrillation protocol used at the Resuscitation Institute and **Figure 4** depicts a representative experiment during the defibrillation phase.
8. Deliver electrical shocks only when VF is present; otherwise resume chest compression without preceding electrical shocks and assume the heart is in pulseless electrical activity or asystole.
9. Determine the resuscitation outcome at the completion of the defibrillation-compression cycles (**Figure 3**).

5.4) Post-resuscitation

1. Increase the ventilation rate from 25 min⁻¹ to 60 min⁻¹ after return of spontaneous circulation and lower the FiO₂ from 1.0 to 0.5 after 15 min of spontaneous circulation.
2. Deliver an electrical shock at the same energy of the last shock if VF recurs. However, VF typically reverses spontaneously to sinus rhythm within a few sec.
NOTE: VF recurrence may occur as part of reperfusion arrhythmias shortly after return of spontaneous circulation but rarely beyond 15 min.
3. Observe the animal according to the specific post-resuscitation protocol decided by the investigator; typically 180 to 240 min in acute experiments without recovery from anesthesia before euthanasia. The timeline of a typical acute experiment is shown in **Figure 5**.
4. Perform necropsy in acute experiments to document position of catheters and injury to internal organs that can render an experiment invalid.
5. Remove all catheters, ligate the vessels, and close the wounds with metal clips and follow the steps listed below in survival experiments.
6. Extubate the animal provided it is able to breathe spontaneously.
7. Return the animal to a clean cage after recovery from anesthesia evidenced by complete and unassisted self-righting from dorsal recumbency.
8. Inject warmed 0.9% NaCl (1 ml/100 g body-weight) intraperitoneally to reduce the risk of hypothermia and dehydration.
9. Administer a subcutaneous dose of meloxicam (2 mg/kg) subcutaneously 4 hours after the dose of analgesia followed by a 1 mg/kg subcutaneous dose once daily for up to 72 hr.
10. House the animal alone with enrichment for up to 48 hr for safer recovery and use the institutional standard operating procedure for post-operative care and monitoring.

Representative Results

The rat model described here was recently used to compare the effects of two inhibitors of the sarcolemmal sodium-hydrogen exchanger isoform 1 (NHE-1) on myocardial and hemodynamic function during chest compression and post-resuscitation⁶¹. It was previously reported that NHE-1 inhibitors attenuate myocardial reperfusion injury by limiting sodium-induced cytosolic and mitochondrial calcium overload, and thereby help preserve left ventricular distensibility during chest compression and attenuate post-resuscitation myocardial dysfunction¹². In this study, the NHE-1 inhibitor cariporide (1 mg/kg), which had been extensively investigated in the past, was compared with the newer compound AVE4454B (1 mg/kg) and vehicle control in three groups of 10 rats each, all subjected to 10 min of untreated VF followed by 8 min of chest compression before delivering electrical shocks. Either compound or vehicle control was randomized for administration into the right atrium immediately before starting chest compression with the investigators blind to the assignment. The effects of the NHE-1 inhibitors were analyzed individually and combined (*i.e.*, against control). As shown in **Figure 6**, NHE-1 inhibition enabled attaining a predefined aortic diastolic pressure (between 26 mm Hg and 28 mm Hg) with less depth of compression consistent with preservation of left ventricular distensibility. When the coronary perfusion pressure was indexed to the depth of compression (CPP/Depth ratio) – an index of left ventricular distensibility – only rats treated with cariporide attained statistical significance. Post-resuscitation, both compounds ameliorated myocardial dysfunction and this effect was associated with greater survival as shown in **Figure 7**. It was concluded based on this study that cariporide is more effective than AVE4454B for resuscitation from cardiac arrest in this rat model.

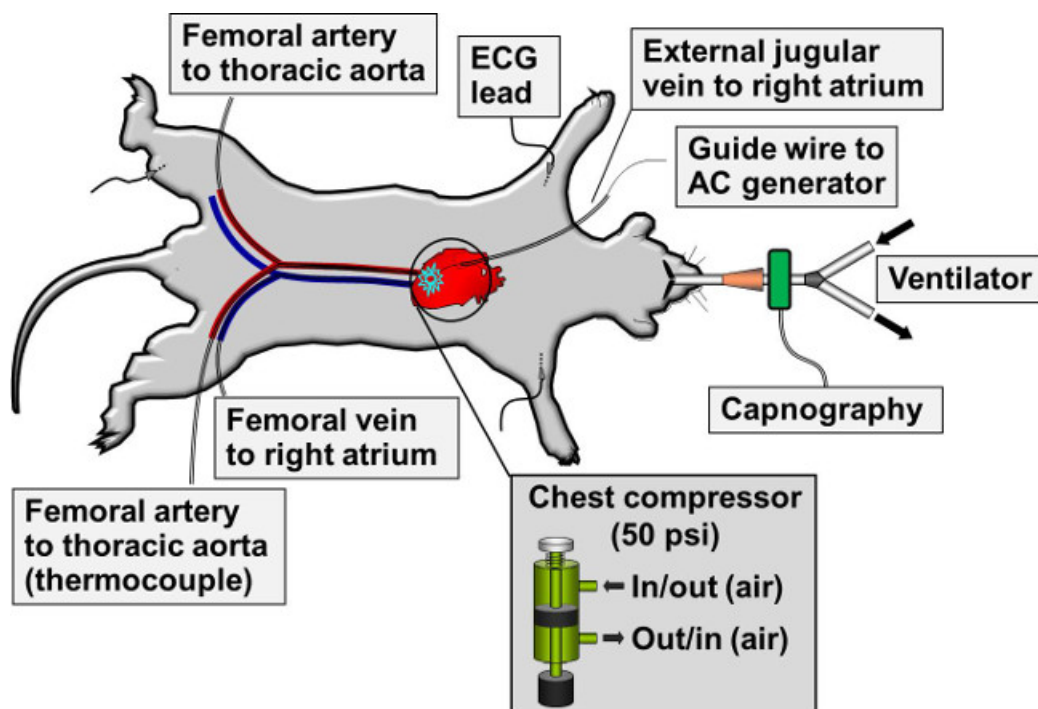


Figure 1: Rat Instrumentation. Schematic rendition of the rat model of VF and closed-chest resuscitation illustrating the various instrumentations and devices used in the model to induce VF and perform cardiac resuscitation. AC = alternating current, ECG = electrocardiogram.

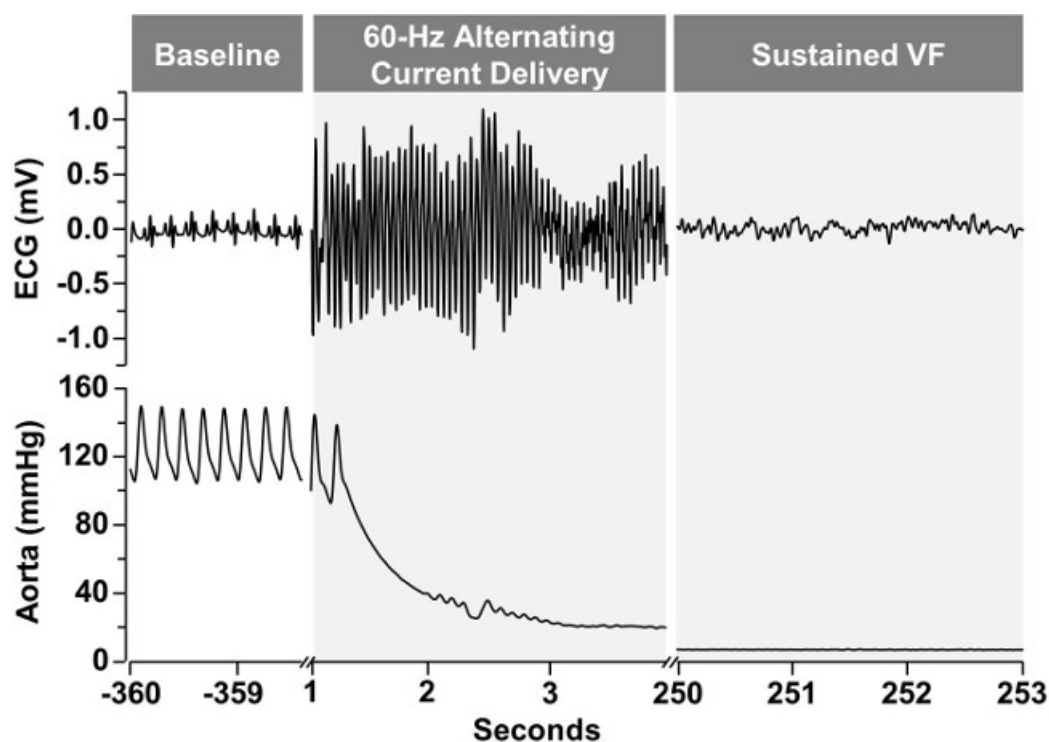


Figure 2: Representative Induction of Ventricular Fibrillation. Experiment depicting the ECG and the aortic pressure at baseline 6 min before inducing VF, at the start of the 60 Hz alternating current delivery to induce VF, and after turning the current off 3 min later. The current delivery typically masks the VF waveform superimposing a 60 Hz waveform, which is no longer seen after turning off the current, documenting sustained VF.

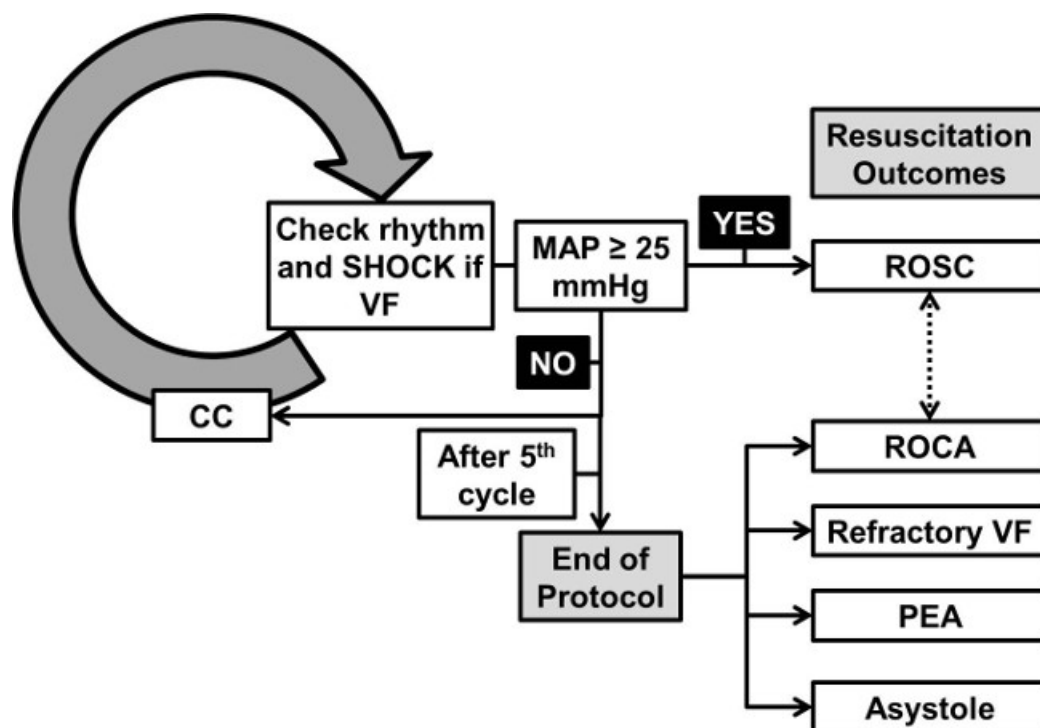


Figure 3: Defibrillation Protocol. Algorithm used to guide when to deliver electrical shocks and when to resume chest compression (CC) based on the electrical cardiac rhythm and the mean aortic pressure (MAP) level. VF = ventricular fibrillation, SHOCK = delivery of electrical shocks. The possible resuscitation outcomes include: (1) ROSC, return of spontaneous circulation defined as a MAP ≥ 40 mm Hg lasting >5 min; (2) ROCA, return of cardiac activity defined as an organized rhythm with an aortic pulse pressure ≥ 5 mm Hg but MAP < 40 mm Hg; (3) refractory VF, defined as the persistency of VF upon completion of the 5th cycle; (4) PEA, pulseless electrical activity defined as an organized cardiac electrical activity with an aortic pulse pressure < 5 mm Hg; and (5) asystole, defined as the absence of electrical and mechanical cardiac activity.

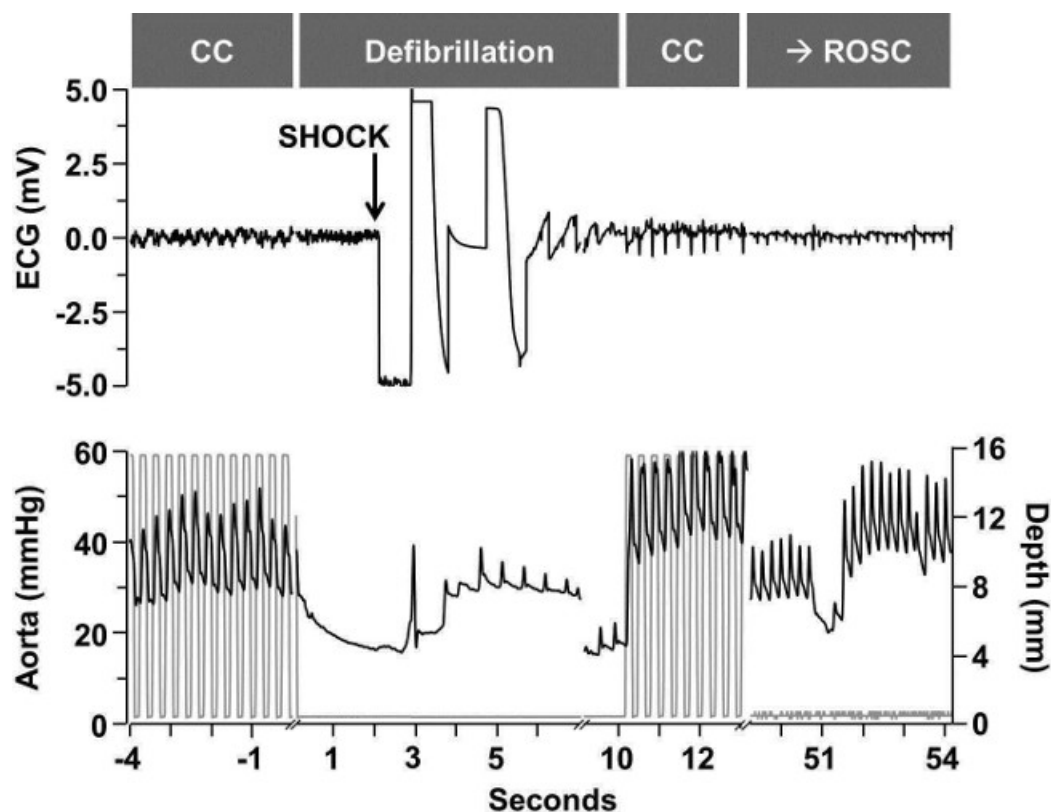


Figure 4: Representative Defibrillation Protocol. Experiment depicting the ECG, the aortic pressure, and the piston displacement (Depth) at the end of chest compression and one additional cycle. Shown are the effects of chest compression (CC) on the aortic pressure while the heart is in VF followed by a pause in chest compression to deliver the initial electrical shock. The shock terminated VF but resulted in weak cardiac activity unable to sustain a mean aortic pressure ≥ 25 mm Hg prompting resumption of chest compression, this time yielding a pulsatile mean aortic pressure >25 mm Hg which rapidly increased to >40 mm Hg consistent with return of spontaneous circulation (ROSC).

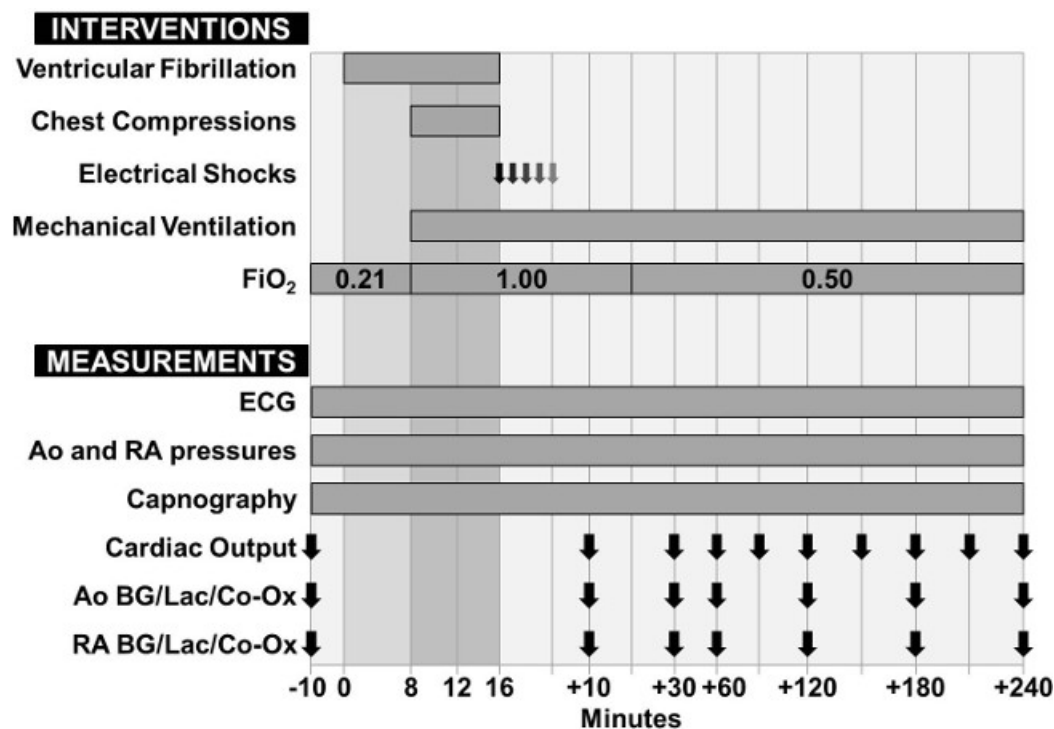


Figure 5: Experimental Timeline. Timeline of a typical acute rat experiment showing interventions and measurements. Ao = aortic, BG = blood gases, Co-Ox = co-oximetry, ECG = electrocardiogram, FiO_2 = fraction of inspired oxygen, Lac = lactate, RA = right atrium.

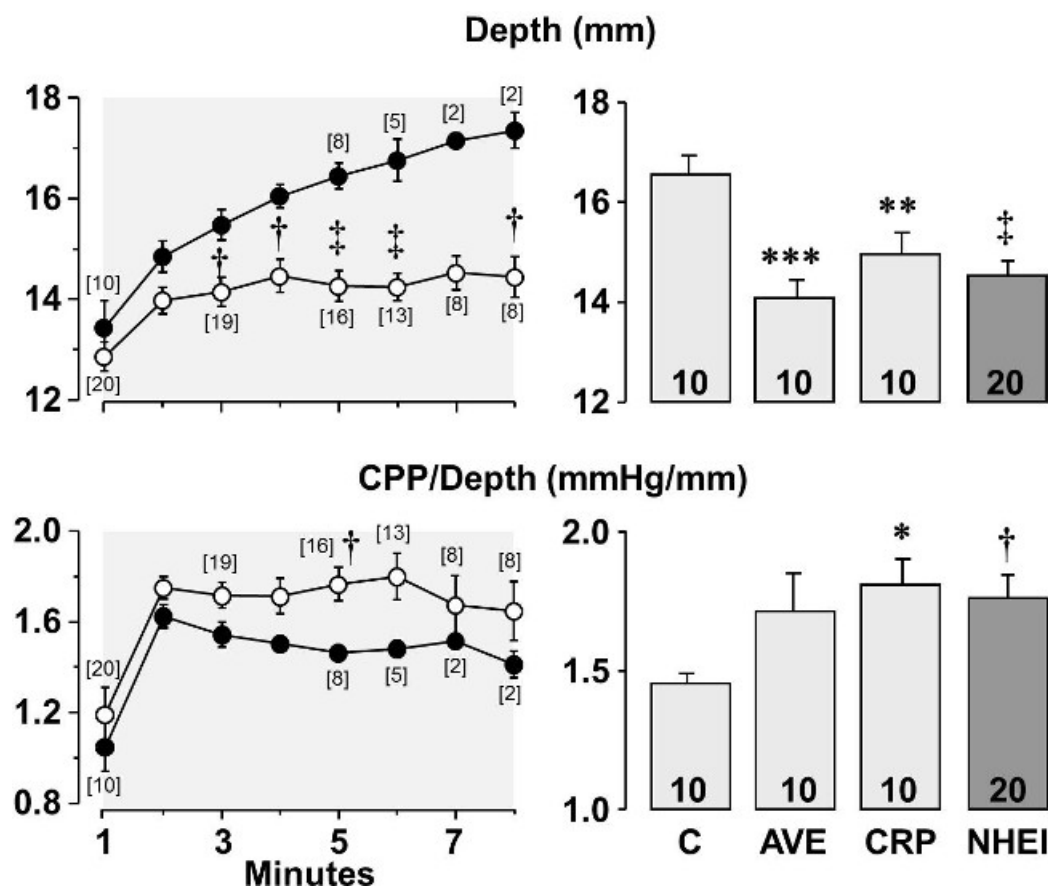


Figure 6: Effect of NHE-1 Inhibitors on CPR Efficiency. The depth of chest compression (Depth) and the ratio between coronary perfusion pressure and depth of compression (CPP/Depth) comparing the control solution (C) with AVE4454B (AVE) and cariporide (CRP) before chest compression. NHEI = AVE and CRP groups combined. Line graphs depict Depth and CPP/Depth throughout chest compression comparing NHEI (○) with controls (●). Numbers in brackets denote rats remaining in ventricular fibrillation. The bar graphs depict the same variables at the last min of chest compression. Values are means $\pm$ SEM. † $p < 0.01$, ‡ $p < 0.001$ vs control by Student's *t*-test; * $p < 0.01$, $p < 0.001$ vs control by one-way ANOVA using Holm-Sidak's test for multiple comparisons; $p < 0.05$ vs control by one-way ANOVA using Dunn's test for multiple comparisons (This figure has been modified from Radhakrishnan *et al.*⁶¹).

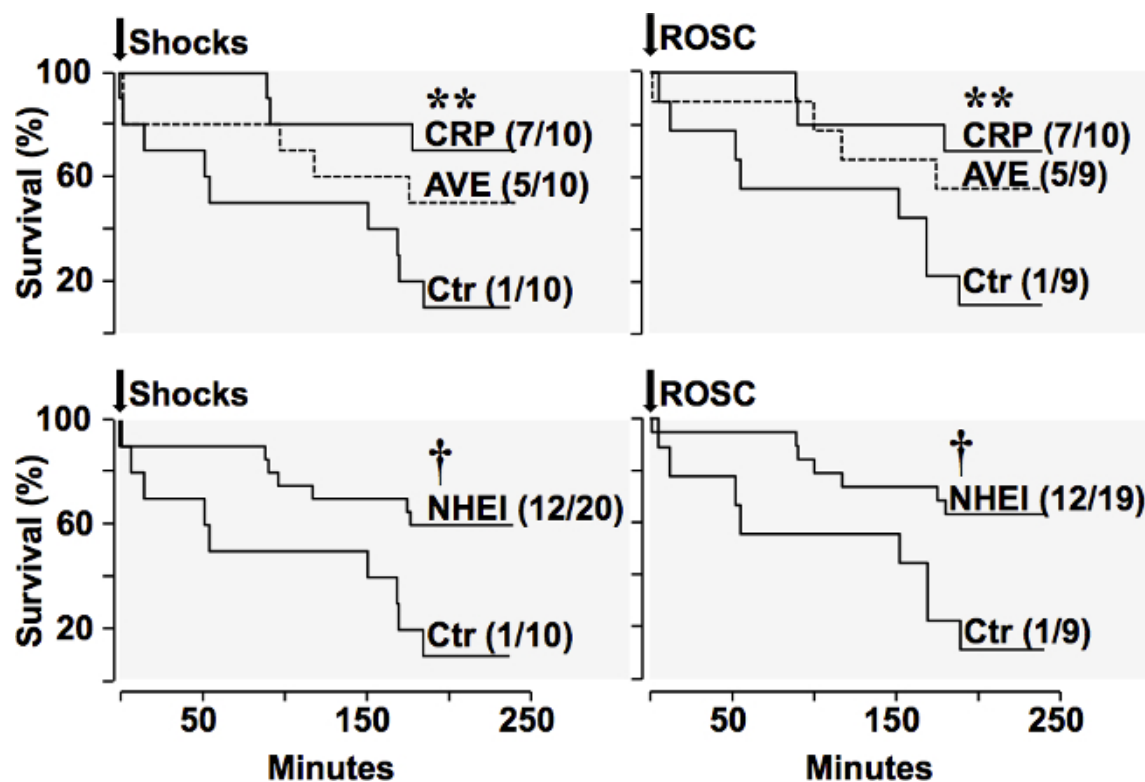


Figure 7: Effect of NHE-1 Inhibitors on Survival. Kaplan-Meier curves in rats that received cariporide (CRP), AVE4454B (AVE), or vehicle control solution. Shown on the left are survival curves for all rats and on the right only those that had return of spontaneous circulation (ROSC). Upper graphs depict survival for the individual interventions and bottom graphs survival for the AVE and CRP groups combined (NHEI). $p < 0.01$ vs control by Gehan-Breslow analysis using Holm-Sidak's test for multiple comparisons; $\dagger p = 0.01$ vs control by Gehan-Breslow analysis (This figure has been modified from Radhakrishnan *et al.*⁶¹).

Variables	Baseline	Post-Resuscitation			
	-5 min	60 min	120 min	180 min	
Temperature (°C)	36.9 ± 0.3 [12]	36.9 ± 0.4 [6]	36.7 ± 0.3 [6]	37.0 ± 0.6 [5]	
HR (min ⁻¹)	379 ± 30	334 ± 27	346 ± 21	370 ± 35	
Cardiac Output (ml/min)	87 ± 13	48 ± 11	33 ± 11	30 ± 10	
Cardiac Index (ml/kg·min ⁻¹)	175 ± 28	93 ± 22	65 ± 20	58 ± 19	
Ao Sysolic Pressure (mmHg)	162 ± 15	108 ± 19	107 ± 24	102 ± 20	
Ao Diastolic Pressure (mmHg)	130 ± 13	84 ± 13	86 ± 21	82 ± 16	
Ao Mean Pressure (mmHg)	141 ± 13	92 ± 15	93 ± 22	89 ± 17	
RA Mean Pressure (mmHg)	0 ± 1	2 ± 1	2 ± 2	1 ± 2	
End-tidal CO ₂ (mmHg)	37 ± 10	34 ± 14	24 ± 16	24 ± 17	
pH, Aorta (unit)	7.40 ± 0.04	7.28 ± 0.11	7.36 ± 0.10	7.34 ± 0.08	
Lactate, Aorta (mmol/L)	0.56 ± 0.32	5.68 ± 2.64	3.24 ± 1.63	3.38 ± 2.15	
PO ₂ , Aorta (mmHg)	84 ± 8	178 ± 18	206 ± 9	206 ± 25	
PCO ₂ , Aorta (mmHg)	40 ± 6	30 ± 11	29 ± 9	24 ± 10	

Table 1: Representative Hemodynamic and Metabolic Values. Baseline values were obtained in 12 male retired breeder Sprague-Dawley rats after completion of surgical instrumentation and before induction of ventricular fibrillation. Subsequent values were obtained at 60, 120, and 180 minutes post-resuscitation. Numbers in brackets denote rats that remained alive in the post-resuscitation interval. Data are shown as mean ± SD. Ao = aortic, HR = heart rate, RA = right atrial.

Discussion

Critical Steps in the protocol

There are critical steps in the protocol. When mastered, the preparation and protocol proceed as succinctly described below. The surgical preparation is expeditious, advancing catheters rapidly through small incisions triggering minimal or no vessel spasm and positioning the catheter tips as intended, followed by successful tracheal intubation after a single or a few attempt(s); thus, completing the preparation in ≈ 90 min from the initial pentobarbital dose to induction of VF with baseline measurements within reference values (**Table 1**). VF is electrically induced in every instance leading to spontaneously sustained VF after 3 min of uninterrupted electrical stimulation in $>95\%$ of the instances. During chest compression, an aortic diastolic pressure ≥ 24 mm Hg and end-tidal $\text{CO}_2 \geq 10$ mm Hg is generated without exceeding a compression depth of 17 mm depth and without injuring intrathoracic organs. Implementation of a defibrillation protocol (e.g., as shown in **Figure 3**) occurs with ease and with <5 sec interruptions in chest compression. Finally, return of spontaneous circulation occurs in $>60\%$ of the experiments using the present protocol or similar ones leading to post-resuscitation myocardial dysfunction with a 240 min survival $>40\%$ and metabolic abnormalities indicative of the systemic oxygen deficit that occurs during cardiac arrest and reverses in the post-resuscitation phase in survivors, as shown on **Table 1**.

Modifications and troubleshooting

The model is highly versatile, allowing for relatively simple adaptations to meet specific research objectives. Recently, use of PE25 size tubing was preferred over PE50 size tubing, which has been used in the past by other investigators, and found it easier to advance into proper position without compromising the fidelity of the pressure measurements. The left ventricle can be catheterized from a carotid artery to assess left ventricular function^{34,61} or to inject microspheres for measuring regional organ blood flow^{6,55}. The trachea may be cannulated directly via tracheostomy instead of the oral – more challenging – technique featured in this article, especially in acute experiments without recovery from anesthesia. Other approaches to induce VF have been described including transcutaneous electrical epicardium stimulation⁷⁴, current delivery to the entrance of the superior vena cava into the heart⁷⁵, and electrical stimulation of the esophagus using a pacing electrode⁷⁶. The method of chest compression may be varied by starting compression at the maximal depth, using lateral restraints, compressing at other rates and duty cycles, and also by using manual technique instead of a piston device. Ventilation can also be varied; the original description used a ventilatory rate of 100 min^{-1} synchronized 1:2 to compressions whereas the present model uses a ventilatory rate of 25 min^{-1} unsynchronized to compressions; consistent with the reduced ventilatory demands of CPR⁷⁷ and current clinical recommendations against pausing for compressions after having established a secured airway. Ventilation can also be passive and promoted by chest compression provided the airway is patent²⁰ or obviated while administering oxygen directly into the trachea²⁵. If an experiment requires removal of large amounts of blood relative to the animal's blood volume $[\text{BV}(\text{ml}) = 0.06 \times \text{body weight (g)} + 0.77]$ ⁷⁸, e.g., for blood collection for determining organ blood flow with microspheres^{6,55} or for repetitive measurement of blood analytes, blood may be transfused from a donor rat from the same colony^{6,55}. Current analytical techniques, however, allow determination of multiple analytes in small samples and administration of equivalent amounts of normal saline or another accepted intravascular solution compensates for small blood losses. The model can also be used to study asphyxia as the mechanism of arrest⁹, which is typically accomplished by inducing neuromuscular blockade and occluding the airway.

Limitations of the technique

The model lacks underlying coronary artery disease and it is technically difficult to acutely induce coronary artery occlusion; conditions most commonly associated with sudden cardiac arrest in humans. The need to maintain the current to induce VF is not ideal and raises concerns of potential injury to the myocardium. Indeed minor thermal injury at the site of current delivery was recognized in the original study, and noted that it could be minimized by reducing the current to the minimum requirement during the 3 min interval needed to induced self-sustained VF⁴. In addition, the electrical current unintentionally triggers skeletal muscle contraction, which could contribute to lactic acid production. The calcium cycling physiology of the rat heart compared to other mammals is less dependent on the sodium-calcium exchanger⁷⁹, and interpretation of related therapies should consider this aspect of the rat cardiac physiology. The rate of compression and ventilation exceeds that used in humans precluding direct extrapolation of related findings. The effects of anesthesia⁸⁰ including cell protective effects⁸¹ should be considered when interpreting findings, although it is not clear that pentobarbital obfuscate findings compared to inhalant anesthetics which have cardioprotective effects⁸¹. Most of the studies reported in the literature have been conducted in male rats intended to minimize possible experimental confounders stemming from different timing within the estrous cycle. Further work is required to assess the effects of gender on resuscitation physiology and outcomes. Another important limitation is the reduced availability of genetically engineered rats relative to mice having to resort to customized genetic engineering or targeted gene manipulation of adult animals through introduction of genetic material (e.g., viral vectors and antisense oligonucleotides).

Significance of the technique with respect to existing/alternative methods

The model is best suited to explore new concepts, new interventions, and to challenge existing paradigms as part of a larger translational strategy that eventually includes focused studies in larger animal models, such as swine, before human trials. Studies in smaller animals (e.g., mice) are complicated by difficulties in inducing VF, limited surgical instrumentation, and the small blood volume that precludes repetitive blood analysis.

Future applications or directions after mastering this technique

The rat model was originally developed to simulate various aspects of human CPR after sudden cardiac arrest. As highlighted in the introduction, the model has been used by investigators to address several aspects of cardiac resuscitation, including its physiology, conventional determinant of outcomes, and mostly the effects of established and novel therapeutic interventions as referenced in this article. The Resuscitation Institute expects the reader to be inspired and use the model to address the many questions in resuscitation research that need further exploration given the disappointing outcomes with current resuscitation methods.

Disclosures

The authors have nothing to disclose.

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