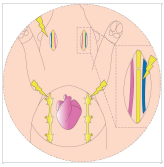

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Evaluating intrinsic cardiac neural control of cardiac function using sequential ganglionated plexus ablations

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DISCLAIMER

ABSTRACT

The purpose of this protocol is to evaluate the control of the intrinsic cardiac nervous system on cardiac function. In a porcine model, autonomic stimulations are performed before and after ablation of cardiac ganglionated plexuses while obtaining hemodynamic and cardiac electrophysiologic recordings.

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KEYWORDS

cardiac autonomic nervous system, cardiac contractility, cardiac electrophysiology, ganglionated plexus, intrinsic cardiac nervous system, ablation

IMAGE ATTRIBUTION

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DISCLAIMER:

Experimental Set-up

1 Pre-sedate a Yucatan minipig via tiletamine-zolazepam (**6 mg/Kg**) intramuscular injection.

2 Sedate pig using isoflurane (5% inhalation, initiation) delivered via nosecone mask. Once loss of blink reflex is confirmed, perform endotracheal intubation using a 7.0mm endotracheal tube. Place pulse oximeter at the lower lip or ear and attach an in-line end-tidal CO_2 monitor.

3 Sedate minipig using isoflurane (1-2% inhalation, maintenance) concomitant with intermittent boluses of fentanyl (up to a total of **20 mcg/Kg** IV).

4 Monitor body temperature using either an esophageal or rectal temperature probe.

5 Continuous 12-lead ECG data are recorded using a Prucka CardioLab (GE Healthcare, Fairfield, CT). Frontal plane lead electrodes are placed in standard positions. To accommodate the open-chest surgical procedure, precordial lead electrodes V1 through V6 are placed posteriorly in the positions of V6 through V11 to mirror standard, anterior precordial lead electrode placement and record the horizontal plane.

6 Additional electrodes may be used to obtain surface ECG in the frontal plane and acquired using CED system and Spike2 software (Cambridge Electronic Design Limited).

7 Obtain vascular access with 7 French sheaths in bilateral femoral arteries for continuous pressure monitoring and 8 French sheaths in femoral veins for fluid and medication administration.

8 Monitor blood gases every 90 minutes using an i-STAT blood analyzer (Abbott, Sylmar, CA). Adjust ventilation as needed to maintain normal pH.

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9 Perform a clamshell thoracotomy to access heart and great vessels.

10 Change anesthesia to α-chloralose intravenous bolus (50mg/Kg) administration followed by continuous infusion at **10 mg/Kg/h** IV. Ensure bolus has completed prior to performing autonomic stimulations below.

11 Perform bilateral neck dissection to access external jugular veins and vagus nerves.

12 Dissect left (LCV) and right cervical vagus nerves (RCV) free, perform bilateral vagotomies, and encircle vagus nerves with platinum nerve cuff electrodes at caudal aspect of vagotomies.

13 Stimulate LCV and RCV individually with nerve cuff electrodes that are connected to a Grass S88 Stimulator (Grass, Warwick, RI) via PSIU6 constant current isolation units. Deliver square wave stimulation pulses (**10 Hz** frequency, **1 ms** duration, **0.1-15 mA**) individually to each vagus nerve.

14 Define stimulus threshold as the stimulation current strength sufficient to elicit a 10% decrease in heart rate.

15 Increase vagal nerve stimulation intensity to 3 times threshold for all subsequent vagal nerve stimulations while maintaining the **10 Hz** frequency and **1 ms** pulse width.

16 Insert a 5 French quadripolar catheter with 5mm interelectrode spacing (Abbott, Sylmar, CA) via the right femoral vein. Advance the catheter to the right atrium.

17 Perform right atrial pacing using a Micropace EPS320 system (Santa Ana, CA).

18 Obtain capture threshold and pace at two times threshold at a rate at least 20% greater than the resting heart rate.

19 Dissect left (LSG) and right stellate ganglia (RSG) free and place either platinum needle electrodes or cuff electrodes between T1-T2 ganglia that are connected to a Grass S88 Stimulator (Grass, Warwick, RI) via PSIU6 constant current isolation units. Deliver square wave stimulation pulses (**4 ms** duration, **4 Hz** frequency, **0.1-15 mA**) individually to each ganglion or sympathetic chain.

20 Define stimulus threshold as the stimulation current strength sufficient to elicit a 10% increase of left ventricular end-systolic pressure (LVESP; for LSG stimulation) or heart rate (for RSG stimulation).

Increase stimulus intensity to 3 times threshold for all subsequent sympathetic stimulations while maintaining the

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21 **4 Hz** frequency and **4 ms** pulse width.

Cardiac Electrophysiologic Recording Set-up

22 Insert a 5 French quadripolar catheter with 5mm interelectrode spacing (Abbott, Sylmar, CA) via the right external jugular vein.

23 Advance the catheter until adequate His signals are captured on the Prucka CardioLab system.

24 Create a pericardial cradle using suture.

25 Connect a custom-made 56-electrode sock placed over both ventricles to the Prucka CardioLab to identify regional activation recovery intervals (ARI).

26 Global ventricular ARIs are calculated via customized software ScalDyn M (University of Utah, Salt Lake City, UT), as described previously.

27 Localized ventricular epicardial activation times (ATs) are measured from the beginning of the QRS complex to the first minimal dV/dt in the QRS complex.

28 Localized epicardial repolarization times (RT) were computed from the beginning of the QRS complex to the first maximal dV/dt of the T wave.

29 ARIs are derived from subtracting ATs from these RTs. This parameter has been shown to correlate with local ventricular action potential durations. Global dispersion in ARI was calculated using the variance of all 56-electrode ARIs.

Hemodynamic Recordings Set-up

30 Assess LV pressures using a Millar Mikro-Tip SPR-350 5 French high-fidelity pressure catheter connected to a PCU-2000 Pressure Control Unit (Millar Instruments, Inc, Houston, TX) placed in the left ventricle via carotid or femoral artery sheath under ultrasound guidance. Data are acquired using the CED system.

Data Collection

31 Perform baseline left and right stellate ganglion and vagal nerve stimulations while performing continuous hemodynamic and cardiac electrophysiologic recordings. Perform vagal nerve stimulations for 25 seconds each with concomitant right atrial pacing during the last 10 seconds of the stimulations. Perform sympathetic stimulations for 30 seconds each.

32 Asses LV systolic function by LV end-systolic pressure (LVESP) and the maximum rate of LV pressure change (dP/dtmax). Assess diastolic LV function by LV end-diastolic pressure (LVEDP) and the minimal rate of LV pressure change (dP/dtmin).

33 Right atrial rate-incremental pacing and progressively premature atrial extrastimuli are delivered using the quadripolar

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catheter inserted via the right femoral vein, and the Atrial-His bundle (AH) intervals and atrioventricular responses are measured to assess atrioventricular nodal conduction.

34 After performing baseline stimulations at 3 times threshold of the bilateral stellate ganglia and cervical vagi, ablate the ganglionated plexi (GPs) on the epicardial surface of the heart using a Stryker Sonopet ultrasonic aspirator (Stryker, Kalamazoo, MI).

a. GPs that are sequentially ablated include: ventral interventricular GP (VIVGP), inferior vena cava-inferior atrium GP (IVC-IAGP) right atrial GP (RAGP), left atrial GP (LAGP) and posterior atrial GP (PAGP).

b. In another cohort of animals, the following sequence of GPs are ablated: RAGP, IVC-IAGP, VIVGP, LAGP and PAGP.

c. In some instances, phenol is applied to the sites of GP ablation to assess completeness of the mechanical ablation.

35 Repeat left and right stellate ganglion and vagal nerve stimulations while performing continuous hemodynamic and cardiac electrophysiologic recordings and pacing maneuvers as above after each GP ablation.

Tissue Harvest for Histological Study

36 To harvest tissues for histologic study at the end of the experiment, a heparin bolus of 5000U IV is administered, and the pig is then placed in ventricular fibrillation with application of a 9V battery to the surface of the heart.

37 The heart is explanted and syringe-flushed with heparinized normal saline (5U/ml) via the transected aorta.

38 The area of interest (RAGP-SAN region) is then excised and rinsed in heparinized saline.

39 Tissue are then immersed in 4% PFA at 4^{o}C with agitation overnight.

40 The following day, the tissues are washed three times for 30 minutes each in 0.01M PBS and stored in either 0.01M PBS and 0.02% sodium azide solution at 4^{o}C for histological study.

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