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Pig-Stellate Stimulation Protocol

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Protocol status: Working

We use this protocol and it's working

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- 1 Animal: Yorkshire Pig

- 2 Surgical Prep:
Sedation Telazol (8mg/kg)
Anesthesia isoflurane (1-2% inhalation) concomitant with intermittent boluses of fentanyl (1–3 µg/kg iv)
- 3 Following completion of the surgery, anesthesia was changed to α-chloralose (50 mg/kg intravenous bolus administration followed by continuous infusion at 10 mg·kg⁻¹·h⁻¹ iv).
- 4 Vascular access: femoral artery (pressure) veins (fluid)

- 5 Mid-sternotomy: access to heart, great vessels
The LSG and RSG were dissected free. and encircled with custom-made semicircular tin-copper electrodes
- 6 Stellate Stimulation
Each left (LSS) and right (RSS) stellate ganglion was stimulated individually via bipolar needle electrodes implanted in the ganglia that were connected to a Grass S88 Stimulator (Grass, Warwick, RI) via PSIU6 constant current isolation units. Square wave stimulation pulses (4-ms duration, 4-Hz frequency, 5-7V) were delivered individually to each ganglion. Stimulus threshold was defined as the stimulation current strength that was sufficient to elicit a 10% increase of left ventricular end-systolic pressure (LVESP) or heart rate. Stimulus intensity was increased to 1.5 times threshold for all subsequent stellate ganglion stimulations, while maintaining the 4-Hz frequency and 4-ms pulse width
- 7 Cardiac Electrophysiology:
ECG recording
Surface ECG recordings. Continuous 12-lead ECG data were recorded using a holter monitoring system (H12digital monitor; Mortara Instruments, Milwaukee, WI). Frontal plane lead electrodes were placed in standard positions. To accommodate the open-chest surgical procedure, precordial lead electrodes V1 through V6 were placed posteriorly in the positions of V6 through V11 to mirror standard, anterior, precordial lead electrode placement and record the horizontal plane. ECGs were analyzed manually. T-wave vector changes in the ventral-dorsal (horizontal plane) and superior-inferior (frontal plane) were assessed using posteriorly placed leads as well as limb leads. Time from peak to end of T-wave (Tp-e) was measured from maximal T-wave voltage to the end of T-wave in limb leads with the clearest T-wave recording
- 8 ARI Recordings
A custom 56-electrode sock, placed over both ventricles, was attached to a Prucka CardioLab (GE Healthcare, Fairfield, CT) to identify regional activation recovery intervals (ARI) .

Global ventricular ARIs were calculated via customized software ScalDyn M (University of Utah, Salt Lake City, UT), as described previously. Localized ventricular epicardial activation times (ATs) were measured from the beginning of the QRS complex to the first minimal dV/dt in the QRS complex. Localized epicardial recovery times (RT) were computed from the beginning of the QRS complex to the first maximal dV/dt of the T wave. Activation recovery intervals were 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 to identify spatial dispersion of regional ventricular epicardial

9 Hemodynamic recording

Systolic LV pressures were assessed by using a 5-F pigtail, 12-pole conductance-pressure catheter connected to an MPVS Ultra processor (Millar Instruments, Inc, Houston, TX) placed in the left ventricle via carotid artery sheath under ultrasound guidance. Proper catheter position was confirmed by the examination of segmental volume signals. Pressure was continuously monitored and recorded throughout experiments. Increases in LV pressures were noted at stimulation onset, confirming successful stimulation capture. Furthermore, a femoral arterial catheter was also placed and used for systemic arterial pressure monitoring.

Total LV volume was calibrated using high-frequency harmonic two-dimensional echocardiographic images (biplane Simpson's method). Hemodynamic indexes were obtained from steady-state pressure-volumen loops. LV performance was assessed by HR, stroke volume (SV), LV end-systolic volume (LVESV), LV end-diastolic volume (LVEDV), cardiac output (CO), and stroke work (SW). Systolic LV function was assessed by ejection fraction (EF), LV end-systolic pressure (LVESP), and the maximum rate of LV pressure change (dP/dt_{max}).

Diastolic LV function was assessed by LV end-diastolic pressure (LVEDP), the isovolumetric relaxation time constant (τ), and the minimal rate of LV pressure change (dP/dt_{min}).

10 Blood gases: monitored.