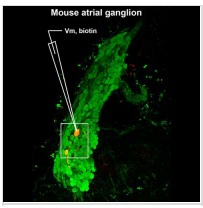

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Protocol for intracellular recording from mouse intrinsic cardiac neurons

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ABSTRACT

Basic protocol for isolation and intracellular recording from mouse intrinsic cardiac neurons is presented. The intrinsic cardiac ganglia, containing hundreds of intrinsic cardiac neurons (ICNs), lie on the dorsal epicardial surface of the mouse heart. These cells receive innervation, principally, from preganglionic parasympathetic neurons of the brainstem by axonal projections within the vagus nerve. These neurons project to multiple targets within the heart (e.g. nodal cells, vascular smooth muscle, myocardium) to affect cardiac dynamics (e.g. heart rate, contractility). This protocol was used for assessing the membrane properties of ICNs sampled from from both control and diabetic mice as presented in the published manuscript (PMID: 31625779) and accompanying dataset.

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KEYWORDS

intracellular recording, intrinsic cardiac neuron, epicardial ganglia, atrial ganglion, diabetes

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Isolation of intrinsic cardiac ganglia

1 Adult mice (M/F; 12±2 weeks of age) are sacrificed under deep isoflurane (5%) anesthesia by cervical dislocation and exsanguination.

2 The thorax is removed and placed in ice-cold physiologic salt solution (PSS) containing in mM: 121 NaCl, 5.9 KCl, 1.2 NaH2PO4, 1.2 MgCl2, 25 NaHCO3, 2 CaCl2, 8 D-glucose; pH 7.4 maintained by 95% O2-5% CO2 aeration.

3 The heart is removed, purged of blood, and pinned to the Sylgard (Dow Corning) floor of a petri dish while continuously superfused with fresh ice cold PSS.

4 Clusters of epicardial neurons in ganglia on the dorsal epicardial surface of the heart, near the pulmonary veins, are visualized with a stereomicroscope and carefully dissected from underlying atrial myocardium using fine forceps (Dumont #7) and iridectomy scissors.

5 The isolated ganglia are pinned to the Sylgard (Dow Corning) floor of a glass bottom petri dish. The dish is secured to a custom XY linear stage and ganglia are observed using an upright microscope (AxioExaminer, Zeiss) equipped with a 5X-dry and a 40X-water-immersion objective, differential interference contrast optics, and monochrome camera (Axiocam, Zeiss).

6 Concentric bipolar stimulation electrodes (FHC) are placed on interganglionic nerves to evoke ortho- or antidromic action potentials at intrinsic cardiac neurons.

Intracellular microelectrode ecording

7 Isolated ganglia are superfused continuously (6–7 ml/min) with PSS maintained at 32–35°C with a thermostatically controlled heater.

8 Neurons are visually identified (40X water-immersion objective) and impaled with borosilicate-glass microelectrodes filled with either 2M KCl (60–120 MΩ) or 2M KCl + 2% Neurobiotin (80-160 MΩ; Vector Labs).

9 Membrane voltage is recorded using a Multiclamp 700B amplifier and headstage connected to a Digidata 1550B data acquisition system.

10 pCLAMP 10 software (Molecular Devices, CA) is used for acquisition and analysis of time series data.

11 Intracellular current injected through the recording electrode is used to characterize membrane physiology.

12 Depolarizing current steps (0.1–0.5 nA, Δ100 pA, 500 ms duration) are used to assess neuronal excitability.

13 Cells are classified as either phasic (<3 APs) or non-phasic (>2 APs) based on the maximum number of action potentials elicited by the depolarizing current.

14 Hyperpolarizing current steps (500 ms) of decreasing amplitude (-0.4 to -0.1 nA, Δ100 pA) are used to test for rectification in the current-induced hyperpolarization, occurring when hyperpolarization-activated currents are initiated, and to measure whole-cell input resistance.

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15 The amplitude and duration of the action potential are measured from either a spontaneous or a nerve evoked spike.

16 After-hyperpolarization amplitude and duration are measured from brief intracellular current pulses (0.1-0.8 nA,Δ100 pA, 5 ms) or spontaneous action potentials.

17 Inclusion criteria for analysis include a resting membrane potential less than or equal to -45mV, a holding current of greater than or equal to -100pA , and the cell must be excitable (cells with no action potential are excluded).

18 Graded stimulus shocks (100 μs) are delivered from the concentric bipolar electrodes in 50-100 μA steps, from 0 to 800 μA, to generate stimulus recruitment curves (Master 8 and IsoFlex optical Isolation unit, AMPI).

19 Five to 20 stimuli are delivered at each stimulus intensity, with an interval of 3 seconds between stimuli.

20 Analysis of synaptic events focuses on latency of the excitatory post-synaptic potential (EPSP), measured from the start of the stimulus trigger to the beginning of the EPSP (V≥2xRMS),and jitter (SD of latency) as indices of conduction, path and release.

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