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Optogenetic Modulation of Cardiac Sympathetic Nerve Activity to Prevent Ventricular Arrhythmias

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ABSTRACT

BACKGROUND Studies have shown that left stellate ganglion (LSG) suppression protects against ventricular arrhythmias (VAs). Optogenetics is a novel technique to reversibly regulate the activity of the targeted neurons.

OBJECTIVES This study aimed to investigate whether an optogenetically silenced LSG could protect against VAs induced by myocardial ischemia.

METHODS Adeno-associated virus (AAV) was used as the vector to deliver ArchT, an inhibitory light-sensitive opsin, to the LSG neurons. Twenty male beagles were randomized into the optogenetics group (n = 10, AAV2/9-CAG-ArchT-GFP microinjected into LSG) and control group (n = 10, AAV2/9-CAG-GFP microinjected into LSG). After 4 weeks, the LSG function and neural activity, heart rate variability, ventricular action potential duration, and effective refractory period were measured in the absence or presence of a light-emitting diode illumination (565 nm). Myocardial ischemia was induced by left anterior coronary artery ligation and 1 h of electrocardiography was recorded for VAs analysis.

RESULTS ArchT was successfully expressed in all dogs. Transient light-emitting diode illumination significantly suppressed the LSG function, LSG neural activity, and sympathetic nerve indices of heart rate variability as well as prolonged left ventricular effective refractory period and APD90 only in the optogenetics group. Thirty-minute illumination further enhanced these changes in the optogenetics group. Importantly, all of these changes returned to baseline within 2 h after illumination was turned off. Moreover, the ischemia-induced VAs were significantly suppressed by illumination only in the optogenetics group.

CONCLUSIONS Optogenetic modulation could reversibly inhibit the neural activity of LSG, thereby increasing electrophysiological stability and protecting against myocardial ischemia–induced VAs. (J Am Coll Cardiol 2017;70:2778–90) © 2017 by the American College of Cardiology Foundation.

Malignant ventricular arrhythmias (VAs) remain a major contributor to sudden cardiac death in patients with myocardial ischemia (1,2). It is well established that the cardiac sympathetic nervous system, particularly the left stellate ganglion (LSG), plays a prominent role in modulating ventricular electrophysiology and arrhythmias (3–5). Left cardiac sympathetic denervation has been applied to treat animals or patients with life-threatening VAs such as post-myocardial

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infarction refractory VAs or electrical storm, long QT syndrome, and catecholaminergic polymorphic ventricular tachycardia (VT) (6–9). Optogenetics is a novel technology that is widely used in the neuroscience field for silencing or enhancing the activity of genetically targeted neurons (10,11). When ArchT, an inhibitory light-sensitive opsin, is genetically expressed in targeted cells and activated by illumination with the appropriate wavelength, it induces hyperpolarizing currents, thus silencing the cells (12–14). In the present study, we aimed to develop a novel optogenetic approach in which an adeno-associated virus (AAV) carrying the ArchT gene was transfected to the LSG neurons. The LSG function and neural activity as well as ventricular electrophysiological properties and VAs in response to myocardial ischemia challenges were evaluated.

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METHODS

ANIMAL PREPARATION. Twenty adult male beagles (body weight 10 to 12 kg) included in this study were supplied by the Center of Experimental Animals in the Medical College of Wuhan University. This study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of Wuhan University. All dogs were anesthetized with Na-pentobarbital (30 mg/kg) and ventilated with room air by a positive pressure respirator (MAO01746, Harvard Apparatus, Holliston, Massachusetts). Additional maintenance doses of 60 mg/h Na-pentobarbital were administered during the procedure. Normal saline at 100 ml/h was infused to replace spontaneous fluid losses. Left femoral artery catheterization was performed to monitor systemic arterial pressure. Body surface electrocardiography (ECG) was recorded with a computer-based Laboratory System (Lead 7000, Jinjiang, Chengdu, China). A heating pad was used to maintain a core body temperature of 36.5 ± 0.5°C. All efforts were made to minimize discomfort.

VIRAL INJECTION INTO LSG. Twenty dogs were randomly divided into the optogenetics group and the control group (Figure 1A). The virus AAV2/9-CAG-ArchT-GFP was chosen for transfecting the LSG. A similar construct (AAV2/9-CAG-GFP) without ArchT was used as a control. The virus was purchased from OBio (Shanghai, China). After anesthesia, a left thoracotomy was performed at the third intercostal space. The LSG was carefully exposed and virus solution (AAV2/9-CAG-ArchT-GFP: 20 μl, 6.25 × 1,012 vector genomes/ml; AAV2/9-CAG-GFP: 20 μl, 5.66 × 1,012 vector genomes/ml) were injected into 4 sites using a 30-G beveled needle (Figure 1B). Virus solution was injected at 1 μl/min, using a 25-μl Hamilton syringe connected to a Harvard PHD syringe pump (Harvard Apparatus). After the injection, the chest was closed in layers and antibiotics (penicillin sodium) were administered for 3 days after surgery.

IMPLANTATION OF THE LIGHT-EMITTING DIODE DEVICE. Four weeks after viral injection, a left thoracotomy was conducted at the fourth intercostal space to expose the heart and LSG. A 2-cm coupled monochromatic light-emitting diode (LED) (565 nm, Convergence Technology, Wuhan, China) was inserted and implanted into the chest wall near the LSG (Figure 1C). The LED was connected externally to the monochromatic LED system (Convergence Technology) for illumination. According to previous studies (15,16), the LSG was illuminated with fixed illumination parameter (40% duty cycle, 20-Hz period, 20-ms pulse width, 3 to 5 mW/mm^2) to reduce heat generation while ensuring the illumination efficiency. All electrophysiological parameters in vivo were measured in the absence or presence of LSG illumination.

MEASUREMENT OF THE LSG FUNCTION. LSG function was assessed by the maximal systolic blood pressure (BP) change induced by high-frequency (HF) stimulation (20 Hz, 0.1-ms pulse duration) using a Grass-S88 stimulator (Astro-Med, West Warwick, Rhode Island) as descried previously (17,18). Because of the variable responses of BP change to HF stimulation in each dog, we therefore applied HF stimulation to the LSG at 4 incremental levels (level 1 = 1 to 4 V; level 2 = 5 to 7 V; level 3 = 7.5 to 10 V; level 4 = 10 to 15 V).

MEASUREMENT OF THE LSG NEURAL ACTIVITY. Neural activity from the LSG was recorded for 1 minute. A tungsten-coated microelectrode was inserted into the LSG, and a ground lead was connected to the chest wall. The electrical signals generated by the LSG were recorded using a Power Lab data acquisition system (8/35, AD Instruments, Bella Vista, Australia) and amplified by an amplifier (DP-304, Warner Instruments, Hamden, Connecticut) with bandpass filters set at 300 Hz to 1 kHz and an amplification range of 30 to 50 times. Neural

ABBREVIATIONS AND ACRONYMS

AAV = adeno-associated virus
APD = action potential duration
APD90 = 90% repolarization duration
BP = blood pressure
ECG = electrocardiography
ERP = effective refractory period
GFP = green fluorescent protein
HF = high frequency
HRV = heart rate variability
LED = light-emitting diode
LF = low frequency
LSG = left stellate ganglion
NGF = nerve growth factor
Smax = maximum slope of the curve
TH = tyrosine hydroxylase
VA = ventricular arrhythmia
VPB = ventricular premature beat
VT = ventricular tachycardia