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.
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.

**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 (MA001746, 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/mm²) 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 described 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
activity, characterized by the recorded amplitude and frequency, was defined as deflections with a signal-to-noise ratio of 3:1 and manually determined as described previously (17–19).

MEASUREMENT OF THE VENTRICULAR EFFECTIVE REFRACTORY PERIOD. Multielectrode catheters were sutured at 3 epicardial sites on the left ventricle. The ventricular effective refractory period (ERP) was determined from the following 3 sites: left ventricular apex, left ventricular base, and the mid left ventricle (the site in the middle of the left ventricular apex and base). The ERP at each site was determined by programmed stimulation consisting of 8 consecutive stimuli (S1-Si, 330 ms cycle length) followed by a premature stimulus (S2). The S1-S2 interval was decreased initially from 250 ms by decrements of 10 ms and then 2 ms until refractoriness was achieved. ERP was defined as the longest S1-S2 interval that failed to capture the ventricles (17,18,20).

MONOPHASIC ACTION POTENTIAL RECORDING. Monophasic action potentials of the left ventricle were recorded from 3 epicardial sites (left ventricular apex, the site in the middle of the left ventricular apex and base, and left ventricular base) using a custom-made Ag-AgCl catheter. A dynamic
steady-state pacing protocol (S1-S1) was performed to determine action potential duration (APD) restitution properties. The pulse train was delivered at an initial cycle length that was slightly shorter than the sinus cycle length, and was maintained for 30 s to ensure a steady state. A 2-min interruption was then taken to minimize the pacing memory effects. Subsequently, another pulse train with the pacing cycle length decreased by 10 ms was delivered until APD alternans appeared. APD alternans was defined as the change in 90% repolarization duration (APD90) ≥10 ms for ≥5 consecutive beats. The monophasic action potential recordings were analyzed by the LEAD 7000 workstation system (Lead 7000, Jinjiang). The APD90 was defined as the 90% repolarization duration, and the diastolic interval was the time interval from the previous APD90 point to the activation time of the next beat. As described in previous studies, the dynamic APD restitution curves were constructed by plotting each APD90 against the preceding diastolic interval using Origin 8.0 (OriginLab, Northampton, Massachusetts). The slope of the shortest diastolic interval was determined: the HF, the low frequency (LF), and the LF/HF ratio (18).

MEASUREMENT OF HEART RATE VARIABILITY. Five-minute electrocardiogram segments were recorded to analyze the spectral power for heart rate variability (HRV) using the autoregressive algorithm. The following power spectral variables were determined: the HF, the low frequency (LF), and the LF/HF ratio (18).

MEASUREMENT OF THE MYOCARDIAL ISCHEMIA-INDUCED VENTRICULAR ARRHYTHMIAS. Myocardial ischemia was induced by left anterior descending coronary artery ligation at approximately 2 cm away from its origin. The ECG during the first hour of ischemia was recorded to analyze the incidence and duration of the VAs according to the Lambeth Conventions. VAs were classified as ventricular premature beats (VPBs), nonsustained ventricular tachycardia (VT), and sustained VT and ventricular fibrillation (18,22).

HISTOPATHOLOGIC STAINING. At the end of the experiment, the LSG was resected for histopathologic staining. Tissues were fixed in 4% paraformaldehyde, embedded in paraffin and sectioned into 4-μm slices. Double immunofluorescence staining was used for tyrosine hydroxylase (TH) (Abcam, Cambridge, United Kingdom) with green fluorescent protein (GFP) (Abcam), c-fos (Abcam), and nerve growth factor (NGF) (Abcam). The nuclei were stained with 4,6-diamidino-2-phenylindole.

STATISTICAL ANALYSIS. All continuous variables were presented as the mean ± SEM and tested for a normal distribution using the Shapiro-Wilk normality test. The continuous variables measured at different time points were compared in the 2 groups (Figures 2 to 5) using 2-way repeated-measures analysis of variance followed by Tukey’s post hoc test. For histologic analysis and VAs (VPB, nonsustained VT), an unpaired Student’s t-test was used to compare the means between the 2 groups. For categorical variables, the incidence of sustained VT and ventricular fibrillation between the 2 groups was compared by Fisher exact test. All data were analyzed using GraphPad Prism software version 6.0 (GraphPad Software, La Jolla, California), and p < 0.05 was considered statistically significant.

RESULTS

VERIFICATION OF ArchT EXPRESSION IN LSG. LSG slices were costained with anti-TH to label sympathetic neurons and with anti-GFP to label GFP/GFP-expression ArchT. The results showed that GFP was mainly expressed in the TH+ neurons in the LSG in both optogenetics and control groups, verifying the successful GFP/ArchT transfection into LSG sympathetic neurons (Figure 1D). Quantification of the overlap between the TH+ and GFP+ cells revealed that 91.82 ± 1.88% of GFP+ cells were TH+ in the optogenetics group and 90.24 ± 1.22% of GFP+ cells were TH+ in the control group (Figure 1E).

OPTOGENETIC MODULATION SIGNIFICANTLY INHIBITED THE LSG FUNCTION. The LSG function (assessed by the maximal systolic BP change in response to LSG electrical stimulation) (Figure 2A) was significantly attenuated by transient and 30 min of LED illumination in the optogenetics group, and the voltage-BP response curve was significantly flattened by transient or sustained optogenetic modulation (Figures 2D and 2E). For example, the maximal systolic BP change during LSG stimulation at voltage of level 4 (10 to 15 V) decreased from 60.9 ± 2.1% to 37.6 ± 1.8% with transient LED illumination and further decreased to 20.0 ± 0.8% after 30 min of illumination. Two h after illumination was turned off, the LSG function recovered to the baseline level before illumination (62.0 ± 2.5%) (Figure 2G). However, no significant changes in maximal systolic BP change or the voltage-BP response curve were observed in the control group (Figures 2B to 2G).

OPTOGENETIC MODULATION SIGNIFICANTLY INHIBITED THE LSG NEURAL ACTIVITY. During illumination (565 nm), the in vivo neural activity recording showed an evident inhibition of LSG neural...
activity by transient optogenetic modulation (Figure 3A). Figure 3B showed representative examples of LSG neural activity recordings at different time points in both groups. Transient optogenetic modulation significantly decreased the frequency and amplitude of LSG neural activity (frequency: 47 ± 4 impulses/min vs. 29 ± 4 impulses/min; amplitude: 0.114 ± 0.012 mV vs. 0.063 ± 0.005 mV; p < 0.05) in the optogenetics group (Figures 3C and 3D). Thirty minutes of illumination further decreased the LSG neural activity (frequency: 11 ± 3 impulses/min; amplitude: 0.015 ± 0.006 mV). Furthermore, the neural activity of LSG recovered to baseline within 2 h after LED illumination was turned off. *p < 0.05 vs. 4 weeks after viral injection; †p < 0.05 vs. transient LED. Abbreviations as in Figure 1.
OPTOGENETIC MODULATION SIGNIFICANTLY STABILIZED THE VENTRICULAR ELECTROPHYSIOLOGIC PROPERTIES. Figure 4A shows representative examples of action potential recordings in the optogenetics group. Transient optogenetic modulation significantly prolonged ERP (Figures 4B to 4D) and APD90 (Figures 4E to 4G) and decreased Smax (Figures 4H to 4J) of the left ventricle in the optogenetics group (Figures 4A to 4I). Thirty-minute illumination further enhanced these changes. Moreover, all these electrophysiological changes returned to the baseline level within 2 h after LED illumination was turned off. Notably, no significant changes in ERP, APD90, or Smax were observed in the control group.

OPTOGENETIC MODULATION SIGNIFICANTLY DECREASED MYOCARDIAL ISCHEMIA–INDUCED VAs. All dogs developed ECG ST-segment elevation or T-wave changes shortly after left anterior artery occlusion. A synchronous LED illumination was performed in the optogenetics group and sustained for 1 h. Acute myocardial ischemia induced VAs (Figure 6A). As compared with the control group, optogenetic modulation significantly decreased the number of VPBs and nonsustained VT in the optogenetics group significantly decreased by either transient or 30-min illumination. Moreover, all these changes returned to baseline within 2 h after illumination (Figures 5A and 5C). However, no significant change was found in the LF or the LF/HF ratio in the control group. Similarly, the optogenetic approach significantly attenuated ischemia-induced increase in the LF and the LF/HF ratio and significantly attenuated ischemia-induced decrease in the HF (Figures 5A to 5C).

(A, B) Representative examples of optogenetic inhibition of LSG neural activity and (C, D) quantitative analysis of LSG neural activity in 2 groups. Transient and sustained LED illumination significantly decreased the frequency and amplitude of LSG neural activity in the optogenetics group. (C, D) The decreased LSG neural activity recovered to baseline within 2 h after LED illumination was turned off. In addition, the ischemia-induced increase of LSG neural activity was also attenuated by optogenetic modulation in the optogenetics group. *p < 0.05 vs. 4 weeks after virus injection. †p < 0.05 vs. transient LED. #p < 0.05 vs. control group. Abbreviations as in Figure 1.
The incidence of sustained VT or ventricular fibrillation in the optogenetics group was also lower than that in the control group (2 of 10 [20%] vs. 7 of 10 [70.0%]; p < 0.05) (Figure 6D).

**OPTOGENETIC MODULATION SIGNIFICANTLY DOWN-REGULATED THE EXPRESSION OF NGF AND C-FOS IN THE LSG.** Figures 7A and 7B show representative examples of double immunofluorescence

**FIGURE 4** Effects of Optogenetic Modulation on Left Ventricular Electrophysiological Properties

![Graphs showing changes in ERP, APD90, and Smax over time with optogenetic modulation.](image)
staining of NGF/c-fos with TH in both groups. Quantity analysis showed that NGF and c-fos in the LSG were significantly more decreased in the optogenetics group than were those in the control group (NGF: 65.2 ± 4.3% vs. 18.9 ± 2.4%, p < 0.05; c-fos: 73.1 ± 4.5% vs. 22.2 ± 2.6%, p < 0.05) (Figures 7C and 7D).

**DISCUSSION**

**MAJOR FINDINGS.** In the present study, we demonstrated an effective and reversible optogenetic modulation to inhibit the LSG neural activity and suppress ischemia-induced VAs. We found that transient LED illumination activated the ArchT pump expressed in the LSG neurons, thereby significantly inhibiting the LSG function and neural activity. The sympathetic component of HRV was suppressed. The stability of ventricular electrophysiological properties, measured by the ERP and APD restitution curve, was enhanced as well. Such effects were further enhanced by 30 min of illumination. Moreover, all the aforementioned changes returned to the baseline level within 2 h after LED illumination was turned off. (A to C) Optogenetic modulation attenuated the ischemia-induced increase in the LF and the LF/HF ratio, and decrease in the HF. *p < 0.05 vs. 4 weeks after viral injection. †p < 0.05 vs. transient LED. #p < 0.05 vs. control group. LED = light-emitting diode.

In view of the important role of cardiac sympathetic nervous system on the genesis and maintenance of arrhythmia, left cardiac sympathetic denervation has been applied to treat post-ischemia refractory VAs or electrical storm, long QT syndrome, and catecholaminergic polymorphic VT (6–9). However, there are some side effects associated with this traumatizing and irreversible operation such as unilateral hand dryness, abnormal sweating, and chest pain (31). In the present study, we applied a...
novel and reversible intervention, optogenetics, to modulate the LSG neural activity. The inhibitory light-sensitive protein utilized in our present study is ArchT, a novel light-driven outward proton pump that is sensitive to green light illumination. When genetically expressed in neurons and activated by LED illumination, ArchT discharges $H^+$ into the extracellular environment, thus causing hyperpolarization and neural silencing (12–14).

Recently, several studies have investigated the inhibitory effect of ArchT on dorsal root ganglion neural activity and neuropathic pain. The results showed that ArchT could be activated in dorsal root ganglion neurons after viral vector administration, thereby inducing a large outflux of $H^+$, producing hyperpolarization, reducing excitability of the opsins-expressed neurons, and reducing mechanical pain hypersensitivity in a neuropathic pain model. Furthermore, activation of ArchT can be turned on or off by adjusting illumination (32–34).

AAV is one of the most widely used gene therapy vectors with stable and safe gene expression, low immunogenicity, and absence of toxicity (35,36). For instance, AAV serotype 9 was effective at transducing the neurons in the enteric autonomic nervous system (37,38). Therefore, we chose AAV2/9 to transfect LSG and deliver ArchT protein into the LSG neurons. Moreover, we directly injected AAV2/9 vectors into the LSG so that the viral infection and ArchT expression could be limited locally in LSG. The results in the present study demonstrated that ArchT was successfully expressed in the LSG, and that viral infection or gene transfer with AAV2/9 did not alter the LSG function and neural activity.

We investigated the effect of optogenetic inhibition of LSG neurons and assessed this inhibitory effect of LSG on ventricular electrophysiology and VAs in normal and myocardial ischemia canines. Direct neural recordings showed that ArchT...
activation by LED illumination significantly inhibited the neural activity of the LSG in normal and myocardial ischemia canines. The LSG function measured by maximal systolic BP change in response to LSG electrical stimulation was also inhibited by ArchT activation. Furthermore, the expression of NGF and c-fos was significantly decreased by sustained ArchT activation with LED illumination, which may underlie the prolonged inhibitory effect of optogenetic modulation on LSG neural activity. These results all imply that activation of ArchT with illumination might result in an inhibition of LSG neural activity, thereby stabilizing the ventricular electrophysiological properties and suppressing the ischemia-induced VAs.

One concern for long-term LED illumination is heat generation. Researches have revealed that an optical power intensity of 10 mW/mm² did not cause detectable temperature changes (16). In view of the great light sensitivity of ArchT, the intensity of light (3 to 5 mW/mm²) used in this study was small to avoid any thermal damage to LSG and collateral tissues. Importantly, the reversibility of the measured LSG function and neural activity as well as the electrophysiological properties after LED illumination was turned off strongly indicates that thermal injury to the LSG neuron plays a negligible role in the findings we observed.

CLINICAL IMPLICATIONS. Most recently, Montgomery et al. (15) and Park et al. (16) reported the development of implantable wireless optogenetic devices that combined tiny soft neural interfaces with fully implantable stretchable wireless radio power and control systems. These tiny, delicate devices can be implanted with minimally invasive techniques and optogenetically modulate the spinal cord and peripheral nervous system. Optogenetics can potentially become a novel minimally invasive patient-controlled technology for treating cardiovascular diseases related to hyperactivity of the cardiac sympathetic nervous system (Central Illustration). For example, the patient may turn on the LED illumination before physical or emotional stress to avoid VAs or defibrillator therapy.
We demonstrated for the first time that optogenetics could reversibly inhibit left stellate ganglion (LSG) neural activity and suppress ventricular arrhythmias (VAs) induced by myocardial ischemia. Wireless optogenetics may become a novel minimally invasive patient-controlled technology for treating cardiovascular diseases related to hyperactivity of the cardiac sympathetic nervous system. LED = light-emitting diode.

STUDY LIMITATIONS. This study investigated the optogenetic modulation of LSG neural activity. As a proof-of-concept study, we only investigated the efficacy of optogenetic modulation by transfecting LSG with AAV at a fixed titer and dosage. Further dose-effect studies are warranted to investigate the correlation between transfection efficiency and neural modulation. Because of the limitation of LED device and neural recording instruments, we could only perform LED illumination and synchronous neural recording under anesthesia. Furthermore, we only made a short-term study of the optogenetic modulation of LSG neural activity and ischemia-induced VAs in an acute myocardial ischemia canine model. Implantable wireless optogenetic devices and neural recording instruments are warranted to investigate the precise control of optogenetics on the cardiac autonomic nervous system and VAs in ambulatory chronic myocardial ischemia models.

CONCLUSIONS

Optogenetic technology could reversibly and controllably modulate LSG neural activity and thus suppress VAs induced by myocardial ischemia. Optogenetics may become a novel minimally invasive patient-activated technology for treating cardiovascular diseases related to hyperactivity of the cardiac sympathetic nervous system.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In a canine model of myocardial ischemia, optogenetic modulation reversibly silenced stellate ganglion sympathetic nerve activity and significantly suppressed VAs.

TRANSLATIONAL OUTLOOK: Further studies are warranted to explore the safety and efficacy of wireless optogenetic modulation of cardiac autonomic innervation and its potential therapeutic utility in man.

REFERENCES


KEY WORDS left stellate ganglion neural silencing, optogenetics, ventricular arrhythmia