The effect of cardiac sympathetic denervation through bilateral stellate ganglionectomy on electrical properties of the heart

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The sympathetic nervous system maintains normal physiological conditions by regulating many homeostatic mechanisms. Abnormal cardiac sympathetic nerve activity contributes to the development of a number of diseases (20, 21, 29), such as catecholamine-sensitive ventricular tachycardias (23, 31), heart failure, and hypertension (5, 6, 10). Pharmacological β-adrenergic receptor antagonists (β-blockers) are widely used to mediate the actions of the sympathetic neurotransmitter; however, they have several limitations due to their global side effects (9, 18) and ineffectiveness in β-blocker-resistant patients.

An alternative to using β-blockers to mediate cardiac sympathetic nerve activity is to surgically remove the stellate ganglia that are the source of sympathetic innervation to the heart. Bilateral stellate ganglionectomy (SGX) denervates the heart by completely removing both left and right ganglia and the connected nerve branches. It has been shown that bilateral SGX leads to a substantial reduction in the neurotransmitter norepinephrine, cardiac epinephrine, and dopamine (32). Although SGX surgeries have been widely accepted, the effect of cardiac denervation on cardiovascular functions of the heart has received limited attention.

Although several studies (1, 32) demonstrated that cardiac sympathetic denervation through bilateral SGX alters cardiovascular functions, its effect on the electrical properties of the heart is still unknown. We hypothesize that bilateral SGX surgery alters electrophysiological properties of the heart, and those changes are facilitated by the downregulation/blockage of the β1-adrenergic receptor. The aim of our study is to characterize the electrophysiological changes caused by a bilateral SGX during periodic pacing and ventricular fibrillation (VF), and to identify possible mechanisms underlying these changes.

MATERIALS AND METHODS

Animal and surgical procedures. Male Sprague-Dawley rats (250 g, Charles River Laboratories, Wilmington, MA) were used for the experiments. All procedures were approved by the University of Minnesota Animal Care and Use Committee and were conducted in accordance with Institutional and National Institutes of Health guidelines.

A total of 44 rats were used: 26 rats (SGX n = 11; sham n = 15) for the optical mapping experiments, 8 rats (SGX n = 4; sham n = 4) for the immunofluorescence analysis, and 10 rats (SGX n = 5; sham n = 5) for the in vivo heart rate measurement. Prophylactic antibiotic (gentamicin sulfate; 10 mg/kg im) was given before each surgery. During the surgeries, rats were anaesthetized with pentobarbital sodium (50 mg/kg) and administered atropine sulfate (0.4 mg/kg) through a single intraperitoneal injection. The rat’s body temperature was maintained at 37°C on a temperature-controlled surgery table.

After the chest and neck were shaved, a polyethylene-190 tube was placed in the rat’s trachea. The tube was connected to a small-animal ventilator (model 683, Harvard Apparatus) with 70 cycles/min and 2.5-ml stroke volume. Surgical denervation of the cardiac sympathetic nerves was achieved by a bilateral SGX, as described previously (32). Briefly, the stellate ganglia located between the first and second ribs beneath the parietal pleura were isolated from their connected nerve branches and extracted. For sham rats, an identical thoracotomy procedure was performed, with the exception of sectioning and removing the ganglia. For both groups, the chest was closed, and negative intrathoracic pressure was restored. The rats were maintained for 8 days postsurgery until the heart was extracted for optical mapping or immunofluorescence experiments.
To perform in vivo heart rate measurements, radiotelemetry transmitters (model TA11PA-C40, Data Sciences, St. Paul, MN) were implanted in the rats, as described previously (32). The radiotelemetry transmitter signal was monitored by a receiver (model RPC-1, Data Sciences) mounted under the rat cage and connected to a data exchange matrix. The heart rate was determined using the commercially available software (Data Sciences). Seven days after the implantation of transmitters, SGX or sham surgeries were performed. The heart rates were collected 8 days after SGX/sham surgeries for 10 s every 4 min for a 25-h period, and average data were calculated.

**Optical mapping.** Rat hearts were quickly removed 8 days post-SGX/sham surgeries by a thoracotomy and immersed in a cardioplegic solution containing the following (mmol/l): 280 glucose, 13.44 KCl, 12.6 NaHCO₃, and 34 mannitol. The aorta was quickly cannulated and perfused with oxygenated Tyrode solution (at 37°C) for ~8 min under constant pressure (70 mmHg) to remove any excess blood from the vessels. The standard Tyrode solution contained (17) the following (mmol/l): 130 NaCl, 1.8 CaCl₂, 4 KCl, 1 MgCl₂, 1.2 NaH₂PO₄, 24 NaHCO₃, and 5.5 glucose (pH 7.4 adjusted with HCl). The hearts were then submerged in a chamber and superfused with Tyrode solution; blebbistatin (10 µmol/l) was then added to the perfusate to reduce motion artifacts. ECG was monitored throughout the entire experiment.

A bolus of 3 ml of the fluorescent voltage-sensitive dye di-4-ANEPPS (10 µmol/l) was injected into the heart. Two diode-pumped continuous excitation green lasers (532 nm, SDL-532-1000 T, Shanghai Dream Lasers Tech., Shanghai, China) were used for excitation, and the fluorescence signal was recorded simultaneously from the right (RV) and left ventricular (LV) epicardial surfaces (>80% of total surface of the heart) by two fast 12-bit charge-coupled device cameras (DALSA, Waterloo, Ontario, Canada). A dynamic pacing protocol was used to periodically stimulate the base of the heart at progressively reduced basic cycle lengths (BCL), from 200 ms in steps of 10 ms, until 90 ms or until VF was initiated. At each BCL, 50 stimuli were applied to reach steady state. Optical movies (3 s) were acquired at 600 frames/s with 64 × 64 pixel resolution (the field of view was 12 × 12 mm²) at steady state of each BCL and during VF. The background fluorescence was subtracted from each frame, and spatial (3 × 3 pixels) and temporal (5 pixels) convolution filters were used.

All experiments in sham and SGX rats were performed under three different conditions: control, isoproterenol (Iso, 10 nM, β-adrenergic receptor agonist) and, β₁-adrenergic receptor antagonist atenolol (Ate, 20 nM). Movies were acquired 20 min after the administration of the Ate, and then fresh Tyrode solution was added to the system for at least 60 min to wash out the drugs. In some experiments (SGX n = 2; sham n = 2), optical mapping moves were acquired after Ate washout. In separate experiments, the effects of the β-adrenergic receptor agonist esmolol (Esm, 6 µM) (7) was evaluated within 9 min (half-life of Esm) of Esm administration on sham rats (n = 3) compared with SGX. Hearts that had not exhibited episodes of sustained VF during periodic pacing throughout the experiment were used for periodic pacing data analysis (SGX n = 4; sham n = 7). The hearts that exhibited sustained VF during control conditions (SGX n = 5; sham n = 7) were used for VF analysis, and all other hearts (SGX n = 2; sham n = 1) were excluded from analysis, since they went to VF after administration of drugs.

**Immunofluorescence.** Eight days post-SGX/sham surgeries, hearts (SGX n = 4; sham n = 4) were fixed in 4% formaldehyde and processed as frozen horizontal sections at a thickness of 10 µm. Ten tissue sections from each heart were harvested from the epicardial surface. The frozen sections were thawed and rehydrated in phosphate-buffered saline. Triton X-100 (0.2%) in phosphate-buffered saline was used to make the tissue cell membrane permeable, and bovine serum albumin (1%) was added to block nonspecific binding sites. The Zenon rabbit IgG labeling kit (Molecular Probes, Eugene, OR) and the rabbit polyclonal β₁-adrenergic receptor antibody (ab3546, Abcam, Cambridge, MA) were incubated on the sections to label β₁-adrenergic receptors. After the sections were washed and fixed for a second time, fluorescent microscopic images were acquired using an upright fluorescence microscope (Axiovert 2, Zeiss, Thornwood, NY). The intensity of the green fluorescence signal indicates the ability of the β₁-adrenergic receptor to interact with the antibody. The relative change in fluorescence signal intensity was compared with the background intensity and was measured and expressed as a percentage.

**Parameter measurements.** Optical action potential durations (APD) were measured at 80% repolarization, and two-dimensional (2D) APD maps were constructed to reveal the spatial distribution of APDs on both LV and RV epicardial surfaces of the heart. Mean APD was obtained at different BCLs for the visible RV and LV surfaces of the heart by averaging APDs from all pixels. The spatial dispersion of APD was estimated based on the heterogeneity index (17) μ = (APD₉⁵ − APD₅)/APD₅₀, where APD₉⁵ and APD₅ represent the 95th and 5th percentiles of the APD distribution, respectively, and APD₅₀ is the median APD distribution.

The changes between mean APDs recorded during control (APDₓ) and Ate (APDₓ,Ate) conditions were calculated as ΔAPD = (APDₓ,Ate − APDₓ)/APDₓ.

APD restitution was constructed for each pixel by plotting steady-state APDs as a function of the preceding diastolic interval (DI = BCL − APD), and curves were fitted using Origin software (Origin-Lab, Northampton, MA) with an exponential function. The maximum slope of the APD restitution curve, S_max, was calculated at the lowest DI. 2D maps of S_max were constructed to reveal its spatial distribution and to calculate the mean value of S_max over the epicardial surface of the heart.

To measure the local conduction velocity, the distributions of activation times for the spatial regions of 5 × 5 pixels were fitted with the plane, and gradients of activation times gₓ and gᵧ were calculated for each plane along the X- and Y-axes, respectively (17). The magnitude of the local conduction velocity was calculated for each pixel as (gₓ² + gᵧ²)⁰. Mean values of conduction velocity were calculated for the RV and LV epicardial surfaces of the heart.

Hearts in which sustained VF (lasting longer than 30 s) was induced by high-frequency pacing under control condition (SGX n = 5; sham n = 7) were used for VF analysis. During sustained VF, optical movies were taken every 10 s, and the first 1,000 frames (1.7 s) of each episode were used for VF analysis. For each VF episode, fast Fourier transform was applied to each pixel to obtain the power spectrum and to determine the distribution of frequencies in the range of 5–35 Hz. The dominant frequency (DF) was defined as the frequency corresponding to the highest peak in the power spectrum (28). 2D DF maps were constructed and used to determine the mean and the maximum DF frequencies on the epicardial surface, as well as the number of frequency domains. The minimum size of the domain was considered to be 30 pixels with a resolution of 0.01 Hz between domains. To reveal singularity points (SP) and to determine the underlying mechanisms of VF, we constructed phase movies, as previously described (11, 15, 33). Briefly, fibrillation was represented by phase values, from −π to π, where excitation, recovery, and diffusion were assigned a different color at each location of the heart. Individual SPs, defined as the point at which all phase values converge, were tracked over 1,000 consecutive frames.

Data are presented as means ± SE. Statistical comparisons between sham and SGX were performed using ANOVA test; the comparison between control and Ate conditions among SGX or sham rats were performed using a paired t-test, and comparison of vulnerability to VF were performed using Fisher’s exact test. P < 0.05 was considered to be statistically significant.
RESULTS

Heart-to-body weight ratio between sham (4.6 ± 0.33 mg/100 g, n = 4) and SGX (4.2 ± 0.54 mg/100 g, n = 4) does not show any significant difference, P = nonsignificant (NS). However, the in vivo heart rate measurements reveal a decrease of average heart rate in SGX (350.74 ± 12 beats/min, n = 5) vs. sham (401 ± 3.7 beats/min, n = 5, P < 0.05).

The effect of SGX on electrical properties of the heart during periodic pacing. Typical examples of 2D APD maps and corresponding traces of action potentials from a single pixel of RV epicardial surface are presented in Fig. 1, B and A, respectively, for sham and SGX at BCL = 90 and 200 ms. Note that the spatial distribution of APDs is similar for both groups; however, the APD of SGX is decreased compared with sham at both BCLs. This reduction occurs predominantly during the plateau phase of the action potential. For instance, at BCL = 200 ms, the mean APD of SGX (n = 4) was 68.1 ± 6.1 ms compared with 100.1 ± 6 ms for sham (n = 7, P < 0.05), and at BCL = 90 ms the mean APD of SGX was 56.8 ± 3.9 ms compared with 69.2 ± 1.5 ms for sham (P < 0.05).

This effect is similar for the LV epicardial surface of the heart, as illustrated in Fig. 1C, where APD values obtained from the LV and RV are compared at BCL 90 and 200 ms. The 2D APD maps of RV and LV epicardial surfaces are shown in Fig. 1D for BCL = 200 ms. Thus there is no interventricular heterogeneity in both sham and SGX at any pacing rate. Therefore, without losing the generality, all further data will be presented for RV epicardial surface of the heart, unless stated otherwise.

Fig. 1. Typical traces of action potential from a single pixel (A) and two-dimensional (2D) action potential duration (APD) maps (B) for sham and stellate ganglionectomy (SGX) at basic cycle length (BCL) of 90 ms (left) and 200 ms (right) under control conditions. Different colors represent different APD values. Single pixel traces in A are taken from pixels denoted as "X" in B. C: mean (±SE) APD values from right (RV) and left ventricle (LV) of SGX (n = 4) and sham (n = 4) at BCL = 90 and 200 ms. D: 2D APD maps from RV and LV surfaces of SGX and sham at BCL = 200 ms.

Fig. 2. Mean (±SE) APDs (A) and mean (±SE) heterogeneity index (B) for RV of sham (n = 4) and SGX (n = 4) at different BCLs. *Significant difference (P < 0.05) between sham and SGX under control conditions. C: typical examples of activation maps for sham and SGX. D: mean (±SE) values of conduction velocity for SGX and sham at BCL = 90 ms.
Figure 2A demonstrates that the mean APDs of SGX are significantly smaller than mean APDs of sham for all BCL values ($P < 0.05$, SGX $n = 4$, sham $n = 7$). To determine whether cardiac sympathetic denervation affects the spatial dispersion of APD, we calculated the $\mu$ at different values of BCLs. Figure 2B indicates that $\mu$ is significantly different between sham and SGX at low pacing rates (BCL = 200 ms) only, but the differences disappear at faster pacing. For instance, at BCL = 200 ms, $\mu = 0.24 \pm 0.03$ (sham) and $\mu = 0.45 \pm 0.06$ (SGX) ($P < 0.05$), whereas, at BCL = 90 ms, $\mu = 0.13 \pm 0.01$ (sham) and $\mu = 0.20 \pm 0.13$ (SGX), $P = NS$.

To investigate whether cardiac sympathetic denervation affects conduction velocity, we constructed activation maps for both SGX and sham, as indicated in Fig. 2C. Both sham and SGX demonstrate a normal propagation of the action potentials from the base, where the pacing sites are located, to the apex of the heart, at BCL = 200 ms. Mean values of conduction velocities (Fig. 2D) indicate no significant differences between sham ($0.67 \pm 0.01$ m/s, $n = 7$) and SGX ($0.71 \pm 0.01$ m/s, $n = 4$, $P = NS$).

To demonstrate the effect of cardiac sympathetic denervation on restitution properties during periodic pacing, we constructed APD restitution curves at each pixel on the epicardial surface, measured the $S_{max}$, and constructed 2D slope maps illustrating its spatial distribution. Figure 3A presents mean APD restitution curves for all SGX ($n = 4$) and sham ($n = 7$). Note that, for both SGX and sham, mean APD decreases as DI decreases, but the APD restitution curve for SGX is more shallow compared with sham. The spatial distributions of $S_{max}$ for both groups are illustrated in Fig. 3B, and the mean values of $S_{max}$ are shown in Fig. 3C. Note the significant reduction of $S_{max}$ in SGX ($0.18 \pm 0.02$) compared with sham ($0.5 \pm 0.05$, $P < 0.05$).

The effect of SGX on the dynamics of VF. In our experiments, 5 out of 9 SGX and 7 out of 15 sham were able to sustain VF (with a duration longer than 30 s) under control conditions, indicating no significant differences in the vulnerability of the hearts to VF.

However, the analysis of VF episodes suggests that the dynamics of VF are different. Figure 4A (top) shows typical examples of DF maps of sham and SGX, indicating the spatial distribution of different frequencies on the epicardial surface of the hearts. The bottom panel of Fig. 4A illustrates typical examples of the time evolution of VF. These traces are taken from single pixels in two spatial locations denoted by “x” in the DF maps. Figure 4B shows a typical example of the frequency spectrogram from a single pixel for both groups. Note the single peak in the sham spectra and multiple peaks in the SGX spectra, suggesting a higher complexity of frequency distribution in the SGX compared with sham rat hearts.

To demonstrate the stability of VF over time, we analyzed VF episodes (1.7 s in duration) and characterized its dynamics at three different time points: 10, 20, and 30 s. Figure 5 illustrates temporal evolution of maximum DF (A), mean DF (B), number of frequency domains (C), number of SP (D), and life span of SP (E) for the epicardial surfaces of sham and SGX. Overall, the maximum DF, number of domains, and number of SPs calculated for SGX (26.45 $\pm$ 1.06 Hz, 12.34 $\pm$ 0.52, and 47.27 $\pm$ 3.40, respectively) are significantly higher than for sham (19.96 $\pm$ 0.59 Hz, 6.36 $\pm$ 0.37, and 19.67 $\pm$ 2.40, respectively, $P < 0.05$), while mean DF and life span of SP are not significantly different between SGX (17.46 $\pm$ 0.76 Hz and 23.98 $\pm$ 1.70 ms, respectively) and sham (14.54 $\pm$ 1.58 Hz and 26.23 $\pm$ 2.18 ms, respectively, $P = NS$). In addition, our data show the absence of interventricular (RV-LV) differences in these parameters, in both SGX and sham (data not shown). Figure 5 also indicates that the dynamics of VF are stable over time: there are no statistically significant differences between these parameters in time during the 30-s period of VF.

The mechanisms of chronic cardiac sympathetic denervation through SGX in the heart. Typical immunofluorescence images of sham and SGX heart tissue sections labeled with R-phycocerythrin are shown in Fig. 6A. The intensity of the green fluorescence signal indicates the ability of R-phycocerythrin to interact with $\beta_1$-adrenergic receptors. Figure 6A shows less fluorescence signal intensity in SGX compared with the sham, suggesting the downregulation/blockage of the $\beta_1$-adrenergic receptor in the SGX. Figure 6B illustrates that the mean relative change of the fluorescence signal intensity compared with the background intensity is significantly lower in SGX ($n = 4$) than in sham ($n = 4$) ($8.9 \pm 1.9$ and 23 $\pm$ 5.1%, respectively, $P < 0.05$).

To further confirm the downregulation of $\beta_1$-adrenergic receptors in SGX, we performed optical mapping experiments using periodic pacing under four different conditions: control, pretreatment with $\beta$-adrenergic receptor agonist Isop (10 nM),...
administration of β1-adrenergic receptor antagonist Ate (20 nM), and washout. Figure 7, A and B, illustrates typical traces of single pixel optical action potentials and mean values of APDs, respectively, obtained from the sham and SGX at BCL = 200 ms under these conditions. Note that Isop does not induce significant changes in APD, while Ate prolongs APD, both in sham (92 ± 8 to 107 ± 9 ms, P < 0.05, n = 4) and SGX (68.1 ± 6.1 to 111 ± 14 ms, P < 0.05, n = 4). Figure 7B also illustrates that washout of the Ate reduces the APDs back to its control values, in both sham and SGX.

The effect of Ate on prolonging APD is different between sham and SGX and depends on pacing rate. Figure 7C illustrates that Ate significantly increases APD in SGX for all values of BCL, while, in sham, the effect is only present at larger BCLs. To quantify the data, we calculated the relative changes of APD due to Ate, ΔAPD, in both groups. Figure 7D shows that ΔAPD is significantly larger for SGX than for sham at all BCLs (P < 0.05, SGX n = 4, sham n = 4).

Finally, we investigated whether β-blockers and cardiac sympathetic denervation have similar effects on electrical properties of the heart. To identify a possible mechanism of SGX surgery, we compared changes in APDs due to SGX surgery with changes in APDs induced by administration of Esm or Ate in sham. Results (Fig. 8) illustrate that both SGX surgery and administration of Esm in sham significantly reduces APDs at all BCLs. For instance, at BCL = 200 ms, the APD of sham control is reduced from 100.1 ± 6 to 68.1 ± 6.1 ms (P < 0.05) or to 83.7 ± 4.6 ms (P < 0.05) due to SGX.
surgery or Esm, respectively. In contrast, administration of Ate in sham increases APDs at larger BCL (at BCL

\[ \text{BCL} = 200 \text{ ms},\] from 94.1 ± 7.7 to 107 ± 9 ms, \( P < 0.05 \)) and has no significant effect at lower BCLs (see BCL = 90 and 140 ms).

**DISCUSSION**

In this study, we detected the effect of cardiac sympathetic denervation by bilateral SGX on electrical properties of the rat hearts during periodic pacing and VF and investigated possible mechanisms underlying these effects.

Our results demonstrate several main findings. First, 8 days of cardiac sympathetic denervation by bilateral SGX shortens APD at all pacing rates and reduces the \( S_{\text{max}} \). On the other hand, cardiac sympathetic denervation does not significantly affect the spatial dispersion of APD. Second, although the vulnerability of sham and SGX to sustained VF is similar, the dynamics of VF are different in these hearts. The maximum DF is higher, and spatial organization of VF is more complex in SGX compared with sham, indicating different underlying mechanisms of VF. Third, we demonstrated that \( \beta_1 \)-adrenergic receptors are downregulated in the SGX compared with sham. Fourth, our data suggest that the mechanism of cardiac sympathetic denervation by SGX surgery is similar to the administration of the \( \beta \)-blocker Esm and different from Ate.

Different methods to achieve cardiac sympathetic denervation. The current mainstays of cardiovascular disease treatments,
β-blockers such as Ate, have been successfully used to reduce the morbidity and mortality in ischemia, heart failure, and hypertension patients (13, 16, 26). β-Blockers can block cardiac β-adrenergic receptors to decrease heart rate and contractility; however, the effects are also prominent in the kidney and brain (9, 10, 18). These global side effects dictate that caution needs to be taken when considering the usage of β-blockers. Another disadvantage is that this approach cannot be used in β-blocker-resistant patients.

SGX produces marked effects on regulating cardiovascular diseases. Cardiac surgeries, such as left cardiac sympathetic denervation, permanently remove the sympathetic innervations to the heart (27) and have been used clinically to reduce the mortality in long-QT syndrome patients who have had failed β-blocker therapy by the removal of left stellate ganglion. Similar effects were also observed in catecholaminergic polymorphic ventricular tachycardia patients (31). Studies show that the removal of the left stellate ganglion modifies two electrophysiological parameters relevant to arrhythmogenesis: ventricular refractoriness and VF threshold. The prolongation of ventricular refractoriness reflects a decreased excitability, whereas a VF threshold increase indicates a significant decrease in the propensity to VF (2, 30). Right ganglionectomy induces long-term heterogeneous alterations of LV electrocardiographic parameters.

Both left and right stellectomy result in only partial cardiac sympathetic denervation compared with bilateral SGX, which might be used for hypertension treatment (1) or for study of chronic cardiovascular diseases (32). In the later study, Yoshimoto et al. (32) demonstrated the reduction of heart rate bilateral SGX rats, despite the fact that the basal mean arterial pressure was not affected. They also showed that Ate resulted in a marked bradycardia in the sham, but had negligible effects on heart rate and mean arterial pressure in SGX.

Cardiac sympathetic denervation and VF. Our results demonstrated that cardiac sympathetic denervation significantly altered electrical properties of the heart. Flattening of APD restitution and decrease of Smax might be an indicator of antifibrillatory effects of the SGX surgery (26, 27). A decrease in Smax is thought to positively correlate with the reduction of the likelihood of wavebreak formation and the destabilization of excitation waves (4, 19). However, in our experiments, both sham and SGX have relatively shallow APD restitution, and, therefore, the direct link between the Smax and vulnerability to VF is not apparent.

Our data demonstrate that, although bilateral SGX does not change vulnerability to VF, it significantly alters the dynamics of VF. The spatial-temporal organization of VF is more complex in the SGX, which is indicated by higher maximum DF, increase in the number of frequency domains, and number of SP over the epicardial surface of the heart. These results suggest that the dynamics of VF are different between sham and SGX and, therefore, suggest potentially different VF treatments. As has been demonstrated previously (3, 33), DF is an accurate and robust estimator of rate of activation during VF, and analysis of optical mapping movies can reveal spatial distribution of local frequencies of excitation in the heart. In addition, SP can occasionally become the center of the rotor, but more often it indicates the fragmentation of a mother rotor wave into daughter wavelets caused by the collision of the excitation front with refractory tails of other waves (3).

It should be noted that changes in electrophysiological properties of the heart induced by bilateral cardiac sympathetic denervation might be different from those induced by the left and right SGX, since, in this case, the sympathetic nerve activities in the heart are not completely eliminated. In addition, our experiments were performed in totally denervated, Langendorff-perfused rat hearts. These issues might be at least partially responsible for the fact that our data indicate no changes in VF vulnerability due to bilateral SGX, although reduced VF inducibility has been implied by previous clinical reports for partial SGX (27, 31). A comparable study of the left/right/bilateral SGX is necessary to address this discrepancy.

SGX surgery shortens APD at all BCL values but does not alter the conduction velocity of impulse propagation in the heart. This suggests that the Na+ current is most probably intact, while calcium transients and K+ channels (in particular transient outward K+ current) might be altered in the SGX heart. Direct imaging using calcium-sensitive dyes is necessary to reveal the actual mechanisms by which SGX alters the electrical properties of the heart.

Mechanism of cardiac sympathetic denervation through SGX surgery. The administration of some β-blockers, such as Ate and sotalol, causes APD prolongation (14, 22), while the opposite effect is observed for β1-adrenergic receptor antagonist Esm (12). Due to these similarities between SGX surgery and administration of Esm, a similar mechanism might be at work in both cases. Therefore, there is a possibility that SGX surgery also downregulates/blocks the β1-adrenergic receptor. To address this issue, we conducted imaging, which shows that SGX has significantly lower fluorescence signal strength and, therefore, indicates the downregulation/blockage of the β1-adrenergic receptor. These results correlate with our optical mapping data showing reduction of APD in SGX compared with sham, and this effect is similar to the blockage of β1-adrenergic receptors with Esm, which acts through inhibition of L-type Ca2+ currents (7). On the other hand, it has been reported (25) that administration of Ate prolongs the APD by competing with the adrenergic neurotransmitters for binding to β1-adrenergic receptors. In this case, the effect of APD prolongation is more pronounced when there are less β1-adrenergic receptors.

It should be noted that the effect in Ate on prolonging APD in SGX hearts is due to the combined effect of bilateral SGX and changes in β-adrenergic receptors. It has been demonstrated previously that the effect of Ate depends on several electrophysiological parameters, such as kinetics of different currents (Na+ current, Ca2+ current, and slow component of delayed rectifier K+ current), the magnitude and time course of intra- and extracellular ion accumulation, etc. (24, 25). In addition, the bilateral SGX might induce additional changes to the heart besides changes in β-adrenergic receptors. Additional studies are necessary to fully understand the mechanisms of bilateral SGX.

Our study demonstrated that SGX surgery shortened the APD and increased the complexity of VF in the heart, and those changes are facilitated by the downregulation/blockage of the β1-adrenergic receptor. Nevertheless, we should keep in mind that this effect could also be due to profound changes in several ionic currents, such as Ca2+ current and transient outward K+ current, that affect the APD and morphology. Therefore, additional studies have to be done to identify the exact mechanism of bilateral SGX.
Limitations. In our study, the SGX and sham hearts were extracted 8 days postsurgery. If animals were allowed to have a longer postsurgery time (longer than 8 days), this might allow the sympathetic nervous system to regenerate the heart due to its property to regenerate. Multiple time point experiments after the SGX surgeries will provide insight into the auto-regeneration process of the sympathetic nervous system.

In our study, we investigated the effect of bilateral cardiac sympathetic denervation on electrophysiological properties of the heart. However, these changes might be different from those induced by the left and right SGX, since, in this case, the sympathetic nerve activities in the heart are not completely eliminated. A comparable study of the left/right/bilateral SGX would be beneficial for understanding the mechanisms of cardiac sympathetic denervation in the heart.

Finally, to eliminate motion artifacts during our optical mapping experiments, we used a small concentration of blebbistatin (10 –15 μM). Although it has been demonstrated (8) that even higher doses of blebbistatin (up to 100 μM) do not affect the APD in rabbit hearts, there is a possibility that blebbistatin might affect the inducibility of VF in the hearts.

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DISCLOSURES
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