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Antiarrhythmic effects of stimulating the left dorsal branch of the thoracic nerve in a canine model of paroxysmal atrial tachyarrhythmias

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Abstract

Background—Stellate ganglion nerve activity (SGNA) precedes paroxysmal atrial tachyarrhythmias (PAT) episodes in dogs with intermittent high rate left atrium (LA) pacing. The left dorsal branch of thoracic nerve (LDTN) contains sympathetic nerves originating from the stellate ganglia.

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Objective—To test the hypothesis that high frequency electrical stimulation of the LDTN can cause stellate ganglia damage and suppress PAT.

Methods—We performed chronic LDTN stimulation in 6 dogs with and 2 dogs without intermittent rapid LA pacing while monitoring the SGNA.

Results—LDTN stimulation reduced the average SGNA (aSGNA) from 4.36 μ V [95% confidence interval, CI, 4.10 to 4.62] at baseline to 3.22 μ V [95% CI, 3.04 to 3.40] after 2 weeks ($P=0.028$) and completely suppressed all PAT episodes in all dogs studied. Tyrosine hydroxylase (TH) staining showed large damaged regions in both stellate ganglia, with increased percentages of TH-negative cells. Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling (TUNEL) assay showed 23.36% [95% CI 18.74 to 27.98] of ganglion cells in the left and 11.15% [95% CI 9.34 to 12.96] ganglion cells in the right stellate ganglia were positive, indicating extensive cell death. A reduction of SGNA and heart rate were also observed in dogs with LDTN stimulation but without high rate LA pacing. Histological studies of the latter two dogs confirmed the presence of extensive stellate ganglia damage, along with high percentage of TUNEL-positive cells.

Conclusions—LDTN stimulation damages both LSG and RSG, reduces left SGNA and is antiarrhythmic in this canine model of PAT.

Keywords

arrhythmia; electrical stimulation; immunohistochemistry; nervous system; autonomic; nervous system; sympathetic

Skin is highly innervated by sympathetic nerves.^{1, 2} In dogs, postganglionic sympathetic nerve fibers of the neck and thorax originate primarily from the stellate ganglia (SG).³ Our recent studies have shown that subcutaneous nerve activity (ScNA) and superficial skin sympathetic nerve activity (SKNA) closely correlate with the stellate ganglion nerve activity (SGNA) in dogs.^{2, 4, 5} The SKNA can also be used to estimate sympathetic tone in humans and is elevated before the onset of atrial and ventricular tachyarrhythmias.^{6, 7} These findings suggest a direct electrical connection between the thoracic subcutaneous nerves and the SG, which in turn controls cardiac arrhythmogenesis. In the central nervous system, prolonged electrical stimulation of the perforant pathway in the rat evokes epileptiform discharges in dentate granule cells and irreversibly damages hilar neurons.⁸ The histological findings, including dendritic and somal degenerative changes, closely resemble the “excitotoxic” type of damage that the putative transmitters glutamate and aspartate are known to cause.⁹ Because there is a direct connection between thoracic subcutaneous sympathetic nerves and the SG, it is possible that rapid and long term electrical stimulation of these nerves can cause SG damage and reduce sympathetic outflow to the heart. Our recent study confirmed that subcutaneous nerve stimulation (ScNS) at two different thoracic sites (Xinshu acupoint and left lateral thoracic nerve) can damage the SG and reduce SGNA in normal ambulatory dogs.¹⁰ It is unclear if ScNS at other thoracic sites can be equally effective in causing SG damage. A spinal nerve is a mixed nerve that carries motor, sensory, and autonomic signals from the spinal cord to the body. The left dorsal branches (dorsal rami) of thoracic nerve (LDTN) refer to the posterior divisions of a spinal nerve that connect to the SG and innervate the muscles and skin of the human back. Those nerves are located under the skin and are easily accessible through a skin incision. The first purpose of the study is to test the hypothesis that

ScNS using LDTN can cause SG damage and reduce SGNA, similar to ScNS using Xinshu acupoint and left lateral thoracic nerve. Rapid intermittent left atrial (LA) stimulation can remodel the atria and cause spontaneous paroxysmal atrial tachyarrhythmias (PAT) preceded by SGNA.^{11, 12} A second aim of the present study is to test the hypothesis that ScNS using LDTN can suppress PAT episodes in dogs with intermittent high rate LA pacing.

Methods

The animal protocol was approved by the Institutional Animal Care and Use Committee. Detailed Methods were included in an online supplement. The study protocols were summarized in Figure 1. At the end of the study, both SG of all dogs were fixed and processed routinely for immunohistochemical staining for tyrosine hydroxylase (TH). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed to probe cell death. The signals were manually analyzed using custom-written software to determine the temporal relationship between nerve activities and heart rate changes. In addition, we also compared the number of PAT episodes over 24 hours between baseline and different time points of the experiment. PAT was defined as an abrupt (>50 bpm/s) increase in the atrial rate to >200 bpm that persisted for at least 5 s.¹² The data were reported as mean $\pm$ Standard deviation (SD) or 95% confidence interval (CI). Paired *t* test and Signed-rank test were performed to compare the differences between heart rate, integrated nerve activities and the number of PAT episodes at different stages of experiments. A two-sided *p* value of 0.05 was considered as statistically significant.

Results

Protocol 1: The effect of LDTN stimulation on PAT

Effects of LDTN stimulation on SGNA and VNA—We found that LDTN stimulation can interfere with the SGNA and VNA similar to that observed during vagal nerve stimulation (VNS) and ScNS from other thoracic sites.^{10, 13} The dogs tolerated 3.5 mA stimulation without showing signs of discomfort or reduced appetite. LDTN stimulation could result in a transient activation of VNA, termination of SGNA, reduction of heart rate (HR) and eliminate the HR variability at the beginning of stimulation (Figure 2A), indicating communication between LDTN, the left SG (LSG) and the left vagus nerve. Figure 2B shows that after 2 weeks of 3.5 mA stimulation, there was a significant reduction of SGNA as compared with baseline, but there was still clear evidence of interaction between the electrical activities of these two structures. These examples also show activation of VNA during LDTN stimulation, coincidental with the occurrence of bradycardia, along with reduced HR variability during sinus rhythm (Figure 2B) and during persistent AF (Figure 2C and D). For all dogs studied, LDTN stimulation reduced the average SGNA (aSGNA) from $4.36 \mu\text{V}$ [95% CI, 4.10 to 4.62] at baseline to $3.28 \mu\text{V}$ [95% CI, 3.02 to 3.54] at one week ($P = 0.027$), and then to $3.22 \mu\text{V}$ [95% CI, 3.04 to 3.40] at 2 weeks ($P = 0.028$).

In the final week of study, immediately prior to tissue harvest, the mean aSGNA was $3.20 \mu\text{V}$ [95% CI, 3.00 to 3.38, $P = 0.028$ compared with baseline] (Figure 3A). However, LDTN stimulation did not significantly change the average VNA (aVNA) or average ScNA (aScNA) as compared with baseline (Figure 3B, 3C, respectively). Figure 3D shows the

mean RR interval increased significantly from 0.58 s [95% CI, 0.53 to 0.62] at baseline to 0.67 s [95% CI, 0.63 to 0.72] at 1 week ($P=0.028$) and to 0.68s [95% CI, 0.62 to 0.74] at 2 weeks ($P=0.028$), indicating that LDTN stimulation can reduce the HR during sinus rhythm. After induction of AF, the RR interval shortened to 0.4 s [95% CI, 0.38 to 0.42 $P=0.028$].

LDTN stimulation reduces PAT Episodes—Consistent with the results of the previous studies,^{11, 12} there were PAT episodes at baseline in the present study (Figures 4A and 4B) and averaged 3 ± 2 episodes per day. However, contrary to those previous studies, no episodes of PAT were observed after LDTN stimulation ($p=0.026$) in spite of 3 ± 1 weeks of intermittent rapid LA pacing. Figures 4C and 4D show that there was HR acceleration during SGNA, but the onset was not abrupt (< 50 bpm) and the maximal HR did not reach 200 bpm. These characteristics failed to qualify that tachycardia episode as PAT. In all dogs studies, none had an episode of tachycardia that reached the threshold for the diagnosis of PAT.

LDTN stimulation Causes SG Damage—All LSG and right SG (RSG) were successfully harvested for analyses. Large areas of damage, characterized by reduced TH staining, pyknotic nuclei and shrinkage of cytoplasm, were visible under low power view in all LSG and RSG (Figure 5A–a, 5A–b) studied. These damaged regions could be either confluent as a large abnormal area or multifocal. The damaged regions had increased percentage of TH-negative ganglion cells (arrows in Figure 5A). The overall mean percentage of the TH negative ganglion cells was 16.15% [95% CI, 14.20 to 18.10] in LSG and 11.58% [95% CI, 10.27 to 12.88] in RSG. In comparison, the normal SG were expected to have only $4.9\% \pm 0.7\%$ of TH-negative cells.¹⁴ Figure 5A–c and 5A–d show high power views of normal and damaged regions, respectively. In the damaged regions (Figure 5A–d), the ganglion cells appeared small, had pyknotic nuclei and stained negatively or weakly for TH. Tissue sections from the same specimens were then double stained for TH and TUNEL. As shown in confocal immunofluorescent images in Figure 5B, the mean percentage of TUNEL-positive ganglion cells was 23.36% [95% CI 18.74 to 27.98] in LSG. TUNEL positive ganglion cells were found in all RSG specimens, with the mean percentage of 11.15% [95% CI 9.34 to 12.96].

Protocol 2: The effect of LDTN stimulation in dogs without rapid atrial pacing

Effects LDTN stimulation on SGNA—We selected the data window in which SGNA was quiescent to examine the effects of LDTN stimulation on SGNA (Figure 6). There was no SGNA response to 0.5 mA stimulus. Increasing the stimulus output resulted in greater SGNA and VNA responses. Red arrows point to significant reduction of the HR during LDTN stimulation when the output increased to 3.5 mA, indicating cardiac effects of LDTN stimulation. At 3.5 mA output, both dogs showed rapid VNA and SGNA activation when LDTN stimulation was given during quiescent periods of SGNA. Red arrows point to reduced HR variability during LDTN stimulation.

Effects of LDTN stimulation on average nerve activities and HR—LDTN resulted in a reduction of aSGNA in both dogs. The aSGNA reduced from 4.05 μ V [95% CI, 3.71 to

4.40] to 3.31 μ V [95% CI, 2.98 to 3.65] after the first week of 3.5 mA LDTN stimulation, and then to 3.21 μ V [95% CI, 2.90 to 3.5] after 2 weeks of 3.5 mA LDTN stimulation (Figure 7A). The mean HR reduced from 95 bpm [95% CI, 90 to 104 bpm] to 80 bpm [95% CI, 73 to 87] (Figure 7D). However, aVNA or aScNA did not show a significant change during monitoring (Figure 7B, 7C, respectively).

LDTN stimulation damages both SG—Bilateral SG of these two dogs were available for analyses. All of them showed large areas of damage visible at low magnification (Figure 8A). Within the left SG, 16.0% [95% CI, 6.72%–25.28%] were negative for TH ($p=0.43$ compared with Protocol 1). In the right SG, 9.27% [95% CI, 0%–19.49%] were negative for TH ($p=0.19$ compared with Protocol 1). The slides from the same specimens were then double stained for TH and TUNEL. Confocal immunofluorescence images (Figure 8B, 8C) showed that abundant TUNEL positive ganglion cells (green) were present in both specimens. In addition, small non-ganglion cells were also found to be TUNEL positive in the same region. The percentage of TUNEL positive cells of these two dogs were 17.49% [95% CI, 0%–46.01%, $p=0.51$ compared with Protocol 1] in LSG and 4.17% [95% CI, 0%–20.75%, $p=0.28$ compared with Protocol 1] in RSG, respectively. These data from Protocol-2 indicate that LDTN stimulation alone without rapid RA pacing can cause SG damage. These findings were consistent with those reported by Yuan et al,¹⁰ who included 8 dogs with ScNS at two different thoracic subcutaneous sites.

Discussion

We found that LDTN stimulation can cause SG damage similar to that induced by stimulating Xinshu acupoint and left lateral thoracic nerve.¹⁰ A second finding is that LDTN suppresses PAT in a canine model of intermittent rapid atrial pacing known to be associated with increased PAT episodes.¹² With the same pacing protocol and methods of analyses, no PATs were observed after LDTN stimulation in the same model.

Mechanisms of SG damage

VNS can cause significant SG damage in dogs.¹⁵ Roughly 1–5% of the cross sectional areas of human and canine vagal nerves are occupied by sympathetic nerve structures.^{16, 17} Stimulation of these sympathetic nerves is likely the reason why SGNA is activated during VNS.^{15, 18} Rapid and prolonged excitation of the SG then causes excitotoxic changes of the SG that is antiarrhythmic.¹⁵ If excitotoxicity underlies the therapeutic effects of cervical VNS, then it follows that stimulating any peripheral sympathetic nerve fibers that originate from the SG should also help control AF through SG damage. Consistent with the latter hypothesis, Yuan et al¹⁰ showed that ScNS at two different thoracic sites could activate SGNA and cause SG damage, resulting in reduced SGNA. In that same study, five control dogs showed no spontaneous SGNA reduction after 6 weeks of observation. LDTN connects to the SG and innervates the skin. The physiological connection between these two structures was proven by observing the effects of LDTN stimulation on SGNA. When the SGNA was active, LDTN stimulation can abruptly terminate the SGNA. On the other hand, when SGNA was inactive (with only baseline activity but no burst discharges), LDTN stimulation consistently induced high amplitude SGNA similar to that observed during

spontaneous burst discharges. Intermittent passive rapid excitation of the SG might then cause excitotoxic type of damages as shown by the histological studies. The SG damage induced by LDTN stimulation is similar to that induced by VNS,¹⁵ suggesting they cause SG damage through the same mechanisms.

Interaction with vagal nerve

In addition to reduction of SGNA, we found that LDTN might directly activate the VNA and produce transient bradycardia. Because vagal nerve is a complex structure¹⁹ that contains both sympathetic and parasympathetic nerves,^{16, 17} it is likely that LDTN stimulation indirectly activated the parasympathetic component of the vagal nerve through the connections in the central autonomic network.²⁰ Both sympathetic withdrawal and parasympathetic activation had contributed to the occurrence of bradycardia during LDTN.

Site of subcutaneous stimulation

Preliminary studies from our laboratory showed that subcutaneous nerve stimulation at two different thoracic sites can damage the stellate ganglion (SG) and reduce stellate ganglion nerve activity (SGNA) in normal ambulatory dogs.¹⁰ The two sites used in the latter study were the subcutaneous nerves at the 5th intercostal space (Xinshu acupoint) and the left lateral thoracic nerve. Both structures contain sympathetic nerve fibers. Stimulating these nerves cause SG damage. The present study used LDTN at the third intercostal space and showed similar damaging effects on the SG. These findings further support the conclusion that stimulating any sympathetic nerves that originate from the SG may cause SG damage. However, there was no aScNA reduction at these stimulating sites. The narrowly spaced bipolar electrodes have failed to record large baseline ScNA. It is thus difficult to demonstrate the ScNA reduction after stimulation. Future studies may need to use a wider spaced bipolar electrodes to monitor ScNA.⁴

Additional mechanisms of SG damage

The LDTN in the third intercostal space is a small nerve. However, the SG damage was quite extensive. Therefore, in addition to excitotoxicity, other factors might also be involved in generating SG damage. Transneuronal (transsynaptic) cell degeneration is a well-documented mechanism of the propagation of neuronal damage in the central and peripheral nervous systems.^{21, 22} We hypothesize that transneuronal degeneration may have played a role in enlarging the area of damage in the SG, thus further the antiarrhythmic effects of LDTN stimulation.

Clinical implications

Neuromodulation methods have been used for the past 40–50 years in the management of patients with atrial and ventricular arrhythmias.^{23–26} More recent clinical trials showed that ganglionated plexi ablation may improve the outcomes of atrial fibrillation ablation.²⁷ Renal denervation, botulinum toxin injection into epicardial fat pads, cutaneous stimulation of tragus and spinal epidural anesthesia have also shown promise in treating patients with arrhythmias.^{28–31} The present study showed that LDTN stimulation may be a useful alternative to the other neuromodulation methods. The skin is easily accessible. Implanting a

subcutaneous neurostimulator does not require thoracotomy, access the vital structures such as vagal nerve, or use of catheters. The device can be easily removed in case of infection or other complications. We propose that the LDTN has a significant potential in treating patients with arrhythmias.

Limitations of the study

We did not include control groups in this study. However, we have previously performed rapid intermittent atrial pacing in 13 dogs and showed the successful induction of frequent spontaneous PATs.^{11, 12} Those dogs serve as the positive control for the present study. In addition, our previous studies showed that SGNA was stable over time in dogs without ScNA.^{10, 32} It is unclear if the SG damage is reversible, or if repeated application of LDTN stimulation beyond the first two months is necessary to maintain antiarrhythmic effects. A third limitation is that LDTN stimulation may result in discomfort or pain. Whether or not humans can tolerate LDTN stimulation is unclear. However, neuromodulation methods have been used extensively in humans to control epilepsy, pain and bladder function. It is likely that LDTN stimulation can similarly be tolerated by humans after prolonged use. Finally, the stimulation parameters have not been systematically evaluated for ScNS. It is unclear if these parameters were optimal for human arrhythmia control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic of the study protocol. **A.** Protocol for LDTN stimulation in a canine model of PAT (n=6). After baseline recording, neurostimulator was turned on (red dot) and programmed 14-s ON (10 Hz, 500 μ s pulse duration) and 66-s OFF for LDTN stimulation. Shaded area indicates that the output current was increased gradually from 0.5 mA to 3.5 mA in 2 weeks. After 2 weeks 3.5mA stimulation, high rate (10 Hz) left atrial pacing was given for 6 days, followed by 1 day of monitoring during which the atrial pacemaker was turned off. Asterisk indicates repeating the latter sequence until persistent (> 48 hours) of AF was induced. **B.** Protocol for LDTN stimulation (n=2) in dogs without rapid atrial pacing. **C.** Anatomical location of LDTN stimulation. a, Black arrow indicates site of LDTN. b, The LDTN. c, The electrodes wrapped around the LDTN.

Figure 2.

LDTN stimulation interacts with SGNA and HR in Protocol 1. The large artifact in SCNA channel indicate the time of ScNS. **A.** shows an abrupt increase of VNA and reduction of SGNA, HR and HR variability during LDTN stimulation. When LDTN stimulation ended, SGNA abruptly resumed. **B** shows after 2 weeks of 3.5 mA LDTN stimulation, SGNA reduced significantly compared to baseline. The onset of LDTN stimulation further reduced SGNA but activated VNA. There was reduction of HR and HR variability during stimulation. C and D are additional examples showing LDTN stimulation may activate VNA and suppress SGNA and HR. (ECG= electrocardiogram, ScNA=subcutaneous nerve activity, SGNA= stellate ganglion nerve activity, VNA= vagal nerve activity, HR= heart rate).

Figure 3.

Changes of nerve activities and RR interval in Protocol 1 (n=6). **A:** aSGNA reduced gradually after LDTN stimulation. The reduction was significant compared to baseline at 1 week (st_1), 2 week (st_2) and the final week of the study. There was a transient increase of aSGNA when rapid atrial pacing was started (pace_1). **B** and **C:** aVNA and aScNA did not change significantly by LDTN stimulation. **D:** RR interval increased gradually after 1 and 2 weeks of LDTN stimulation. Rapid atrial pacing induced non-sustained AF and then persistent AF, which are associated with reduced the average RR interval in the final week of the study.

Figure 4.

Typical examples of paroxysmal atrial tachyarrhythmia (PAT) episodes. **A** and **B** show PAT episodes at baseline. Arrows point to abrupt onset (> 50 bpm increase). The PAT episodes were typically associated with burst SGNA. **C** and **D** show typical episodes of HR response to SGNA after a period of LDTN stimulation. Note that there were transient elevation of HR. However, these were not counted as PAT episodes because the rate was < 200 bpm and the onset was not abrupt (< 50 bpm).

Figure 5.

Bilateral SG damage induced by LDTN stimulation. **A:** Tyrosine hydroxylase (TH) staining showing damaged areas at low magnification in both LSG and RSG. In the normal regions, the ganglion cells showed normal morphology and positive TH staining, while the ganglion cells appeared small (red arrows), had pyknotic nuclei and stained negatively or weakly for TH in the damaged regions (black arrows). **B** and **C:** Confocal images of Tyrosine hydroxylase (TH, red) and TUNEL (green, yellow arrows) double staining. Blue is the DAPI stain of the nuclei. TUNEL positive ganglion cells (yellow arrows) were present in both LSG and RSG. Some non-ganglion cells were also TUNEL positive.

Figure 6.

LDTN stimulation activates SGNA in Protocol-2 dogs. LDTN artifacts were clearly visible on ScNA channels. There were no SGNA response to 0.5 mA LDTN stimulation in either dog. Increasing the output to 1.5 mA, 2.5 mA and 3.5 mA resulted in increased responses along with reduced HR and HR variability (red arrows). There was VNA elevation during the ON-time of the LDTN stimulation in both dogs. These data indicate a physiological connection between LDTN, the SG and the vagal nerve.

Figure 7.

Change of nerve activities and heart rate in Protocol 2 (n=2). **A:** LDTN stimulation started resulted in a reduction of aSGNA in both dogs at first week (Sti_1) and second week (St_2) stimulation at 3.5 mA. **B** and **C** show no significant changes of aVNA and aScNA during the same time period. **D** shows heart rate changes after the onset of LDTN stimulation.

Figure 8.

Histology results of bilateral SG in Protocol 2. **A:** Tyrosine hydroxylase (TH) staining showed visible damaged areas under low power view in both LSG and RSG. In the normal regions, the ganglion cells showed normal morphology and positive of TH staining, while the ganglion cells appeared small (red arrows), had pyknotic nuclei and stained negatively or weakly (black arrows) for TH in the damaged regions. **B** and **C:** Confocal images of Tyrosine hydroxylase (TH, red) and TUNEL (green, yellow arrows) double staining. Blue is the DAPI stain of the nuclei. Damaged ganglion cells were present in both LSG and RSG. The damaged ganglion cells stained positive for TUNEL and either positive or negative for TH (yellow arrows).