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Original Article

Atrial sympathetic and parasympathetic nerve sprouting and hyperinnervation induced by subthreshold electrical stimulation of the left stellate ganglion in normal dogs

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Abstract

Background: Subthreshold electrical stimulation of the left stellate ganglion (LSG) can induce nerve sprouting and sympathetic hyperinnervation in canine ventricles. It is unclear whether a similar neural plasticity involving both sympathetic and parasympathetic innervation also exists in the atria. **Methods and Results:** We applied subthreshold electrical stimulation at 20 Hz (0.45 ms pulse width) or 5 Hz (1.9 ms pulse width) to the LSG in 6 normal mongrel dogs. After 41±9 days, the hearts were harvested and the right and left atrium stained for synaptophysin (SYN), growth-associated protein 43 (GAP43), sympathetic nerve markers tyrosine hydroxylase (TH), and parasympathetic marker choline acetyltransferase (ChAT). Tissues from 6 additional healthy dogs were used as controls. The hearts from dogs with LSG electrical stimulation had a higher density of nerve structures immunopositive to the SYN, GAP43, TH, and ChAT ($P<.01$) in both right and left atria. Nerve density was equal in right and left atria. There were more TH-positive nerve structures than ChAT-positive nerve structures ($P<.01$) for both right and left atria. No atrial arrhythmia was observed at the second surgery. **Conclusions:** Continuous subthreshold electrical stimulation to the LSG induces both sympathetic and parasympathetic hyperinnervation in both right and left atria in normal dogs. © 2008 Published by Elsevier Inc.

Keywords: Electrophysiology; Nervous system; Nerve sprouting; Pacing

1. Introduction

Long-term potentiation (LTP), a use-dependent form of long-lasting enhancement of synaptic efficacy, has been widely studied in the central nervous system and considered a mechanism for learning and memory [1]. LTP is also thought to be one of the cellular mechanisms underlying the kindling model of epilepsy. Kindling stimulation to the perforant path is associated with progressive axonal sprouting and synaptic reorganization in mossy fibers that might be the anatomic substrate for the increased neuronal hyperexcitability [2]. It is possible that similar neural plasticity, namely, axonal sprouting to electrical stimulation, also exists in the peripheral autonomic nervous system. Consistent with this hypothesis, we [3] have demonstrated that subthreshold electrical stimulation of the left stellate ganglion (LSG) can cause ventricular sympathetic nerve sprouting and hyperinnervation. We [4,5] have also demonstrated that electrical stimulation to the right atrium (RA) and left atrium (LA) that contained the postganglionic sympathetic nerve terminals induced localized sympathetic hyperinnervation near the stimulation site. The ganglion cells of ventricular nerves are located in the stellate ganglion [6]. Therefore, it is not surprising that electrical stimulation of the LSG can cause

Abbreviations: LTP, long-term potentiation; LSG, left stellate ganglion; NGF, nerve growth factor; TH, tyrosine hydroxylase; SYN, synaptophysin; GAP43, growth-associated protein 43; ChAT, choline acetyltransferase.

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nerve sprouting in the ventricles. In contrast, there is a large number of ganglion cells within the atrial itself [6]. Electrical stimulation might have caused nerve sprouting in atria by stimulating those ganglion cells. However, whether or not electrical stimulation of LSG can induce atrial nerve sprouting remains unclear. Previous studies have indicated that simultaneous sympathetic and parasympathetic activation are highly arrhythmogenic in the atria [7]. A likely mechanism is that sympathetic stimulation increases intracellular calcium while parasympathetic stimulation shortens action potential. When these two arms of the autonomic nervous system activate together, it might predispose that atria and pulmonary veins to after-depolarizations and triggered activity [8,9]. Furthermore, Inoue and Zipes [10] showed that simultaneous sympathetic and vagal stimulation on the right atrial refractoriness was not only additive but rather synergistic. Massively shortened refractoriness can also promote reentry formation. We [11] have demonstrated that subthreshold LSG stimulation may facilitate the development of paroxysmal atrial fibrillation in diseased canine hearts. We hypothesize that, in addition to inducing ventricular nerve sprouting, subthreshold electrical stimulation of LSG can also induce nerve sprouting in the atria and that the nerve sprouting involves both parasympathetic and sympathetic nervous systems. The purpose of this study was to test these hypotheses.

2. Methods

The research protocol was approved by the Institutional Animal Care and Use Committee of the Cedars-Sinai Medical Center and followed the guidelines of the American Heart Association. The effects of subthreshold electrical stimulation on ventricular nerve sprouting in these same dogs have been reported in a previous publication from our laboratory [3]. Because that study focused on the induction of ventricular arrhythmia by subthreshold electrical stimulation of the LSG, the data on atrial nerve sprouting were not included in that report. Furthermore, we did not evaluate the parasympathetic nervous system in that report. Because simultaneous sympathetic and parasympathetic activation was recently proposed as important mechanisms that trigger atrial fibrillation [9], we thought it is worthwhile to analyze the atrial nerve density retrospectively in those normal dogs to determine if LSG stimulation alone (without myocardial infarction or atrioventricular block) can induce both sympathetic and parasympathetic hyperinnervation in the atria.

2.1. Subthreshold electrical stimulation of LSG in normal dogs

The surgical procedures have been previously reported in detail [3]. Briefly, six normal dogs (21–28 kg) were anesthetized with isofluorane. The chests were opened via the left fourth intercostal space. We first defined LSG stimulation threshold for each dog by stimulating the LSG at 20 Hz (5 ms pulse width) using a Bloom constant current isolator. The electrical current (mA) strength that elicited an abrupt increase of systolic blood pressure and a heart rate of more than 20% from the baseline was defined as stimulation threshold. An active fixation pacemaker lead was then secured to the LSG and connected to a Medtronic Itrel neurostimulator (n=3) or a modified Guidant Discovery pacemaker (n=3) to give rapid stimulation at 20 Hz (0.45 ms pulse width) or 5 Hz (1.9 ms pulse width), respectively. The actual pacing time was almost the same for both 20- and 5-Hz pacing methods (9 ms/1 s vs. 9.5 ms/1 s, respectively). Because both pacemakers were constant-voltage devices and the pacing lead impedance may vary between 500–1000 Ω, we multiplied the current threshold by 1000 to estimate the voltage threshold for LSG stimulation. We then programmed the pacemaker output to 25% of the calculated voltage threshold and monitored the blood pressure and heart rate again to confirm that this output was indeed subthreshold. The chest was closed, and the animals, while ambulatory, were stimulated with subthreshold current to the LSG continuously for 30–53 (41±9) days.

2.2. Second surgery

At the completion of LSG stimulation, the dogs were anesthetized with isofluorane. The chests were opened via the left fourth intercostal space and the heart was quickly removed. The heart was fixed in 4% formalin for 1 hour and preserved in 70% ethanol [12]. The tissue sections were paraffin embedded and processed routinely for immunohistochemistry analyses.

2.3. Immunocytochemistry

The nerve markers tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), synaptophysin (SYN), and growth-associated protein 43 (GAP43) were immunostained on 5-μm transmural sections using a modified immuocytochemical ABC method [12–14]. The control tissues were obtained from right and left atria of six normal healthy mongrel dogs. The primary antibodies used in this study were monoclonal mouse antirat TH (Boehringer Mannheim Biochemica, Indianapolis, IN; working concentration, 0.2 μg/ml), polyclonal antihuman ChAT (1:50 dilution, Chemicon), rabbit antihuman SYN (DAKO, dilution 1:100), and monoclonal mouse antirat GAP43 (Boehringer Mannheim Biochemica).

2.4. Nerve density measurement

We took three samples each from appendage and free wall of both atria. Therefore, 12 sections were taken per animal. After staining, for each slide, five fixed fields (right upper, right lower, left upper, left lower, and middle) were examined under microscope to determined the nerve

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