Low-level carotid baroreflex stimulation suppresses atrial fibrillation by inhibiting left stellate ganglion activity in an acute canine model

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BACKGROUND Low-level carotid baroreflex stimulation (LL-CBS) appears to have a potential antiarrhythmogenic effect.

OBJECTIVE The purpose of this study was to investigate effects of short-term LL-CBS on an atrial fibrillation (AF) canine model.

METHODS Group 1: Anesthetized dogs underwent 6 hours of rapid atrial pacing (RAP) with concomitant LL-CBS in last 3 hours (LL-CBS group; n = 7) or without (control group; n = 6). Effective refractory period (ERP), ERP dispersion, and window of vulnerability to AF were determined. Left stellate ganglion (LSG) neural activity and heart rate variability were analyzed. Group 2: In subgroup 1, sustained AF was induced by injecting acetylcholine (Ach; 10 mM) into the anterior right ganglionated plexus at baseline and after 3-hour LL-CBS (n = 7) or sham operation (n = 6). In subgroup 2, Ach was applied onto the right atrial appendage. The time of duration of AF and the average AF cycle length were determined in both subgroups.

RESULTS Group 1: LL-CBS reversed the RAP-induced ERP shortening and increase in ERP dispersion and window of vulnerability ($P < .05$). The activation of LSG, decrease in high frequency, and increase in low frequency and low frequency/high frequency ratio induced by RAP were also reversed by LL-CBS ($P < .05$). After 6-hour RAP, plasma norepinephrine and angiotensin II concentrations were significantly lower in the LL-CBS group than in the control group ($P < .05$). Group 2: The AF duration was shortened and the average AF cycle length was prolonged markedly in both subgroups ($P < .01$) by LL-CBS.

CONCLUSION LL-CBS can reverse RAP-induced atrial electrical remodeling and suppress electrically or mechanically induced AF with Ach, and the anti-AF effect is attributed to attenuation of autonomic nerve remodeling, including inhibition of the LSG activity.

KEYWORDS Low-level carotid baroreflex stimulation; Atrial fibrillation; Left stellate ganglion; Autonomic remodeling; Autonomic nervous system

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia that results in significant morbidity and mortality.[1] Antiarrhythmic drugs, electrical cardioversion, and catheter ablation techniques have been used to treat AF, while clinical outcomes are far from satisfactory. In recent years, the autonomic nervous system was validated to play an important role in the initiation and maintenance of AF.[2] Varieties of interventions on the modulation of the autonomic nervous system have shown potential for AF control.[3]

Carotid baroreflex stimulation (CBS) modulates the autonomic nervous system by sympathetic suppression as well as vagal activation.[4] Recent studies found that moderate CBS that decreased blood pressure (BP) and heart rate showed proarrhythmic effects including shortening of the effective refractory period (ERP).[5] In contrast, our recent studies demonstrated that low-level CBS (LL-CBS) without BP or heart rate reduction exhibited antiarrhythmic potential. LL-CBS prolonged ERP and monophasic action potential duration of the left atrium in rabbits and suppressed local AF inducibility by high-frequency (HF) stimulation during the

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refractory period in canines.[6,7] In this study, we further investigate the effects of LL-CBS on AF induced by different methods and its underlying mechanism.

Methods

The detailed methods are described in the Online Supplement.

Animal preparation

All animal studies were reviewed and approved by the ethics committee of the Renmin Hospital of Wuhan University and followed the guidelines outlined by the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health. Thirty-eight adult mongrel dogs (weighing 16–20 kg) were anesthetized with Na-pentobarbital (30 mg/kg) and ventilated with room air by a positive pressure respirator (MAO01746, Harvard Apparatus, Holliston, MA). Normal saline at 50–100 mL/h was infused to replace spontaneous fluid losses. Core body temperature was maintained at 36.5° C ± 1.5°C. Standard surface electrocardiograms and BP were monitored continuously. The depth of anesthesia was monitored by checking heart rate, breathing rate, and the toe-pinch response. The procedures of animal preparations and bilateral thoracotomy as well as positions of electrodes at multiple atrial and pulmonary vein (PV) sites have been communicated in detail elsewhere (Figures 1A and 1B).[8] All recordings were displayed on a computer-based, electrophysiology system (LEAD 2000, Jinjiang, Inc, XXXX, China).

LL-CBS

We have previously described the methods for LL-CBS.[6] Electrodes were positioned once the location was identified in which stimulation (frequency 20 Hz; stimulus duration 2 ms) of >5 V could elicit an abrupt decrease of >10% in systolic BP from baseline (Figures 1C and 1D). The intensity of LL-CBS was set to 80% of the threshold (V) to cause systolic BP reduction. The threshold was reassessed every 1 hour to adjust the voltage for LL-CBS.[6]

Study protocol 1: LL-CBS in the 6-hour RAP model

Group 1 (n = 14): After determination of the baseline values of ERP and WOV, rapid atrial pacing (RAP) was delivered at 1000 beats/min (2 × threshold) at the left atrial appendage for 6 hours. After each pacing hour, RAP was temporarily stopped for 10–15 minutes to measure the ERP and AF inducibility. LL-CBS was applied concomitantly during the last 3 hours of the 6-hour RAP in the LL-CBS group (n = 8). Six other animals that underwent 6-hour RAP without LL-CBS served as the control group. During ERP measurements, AF was induced by the S1-S2 protocol. The difference between the longest and the shortest S1-S2 interval that induced AF was defined as the *window of vulnerability* (WOV). We chose ΣWOV as the quantitative measurement of AF inducibility of the whole heart, which was considered as the sum of WOVs at all 7 recording sites. Before RAP and after 3-hour and 6-hour RAP, left stellate ganglion (LSG) neural activity was recorded (Figures 1E and 1F) for 15 minutes and heart rate variability (HRV) power spectral was analyzed. After 3-hour and 6-hour RAP, 5 mL venous blood was collected (Figure 1G) for plasma norepinephrine (NE) and angiotensin II (Ang II) tests. LSG samples of all animals were obtained from the recording sites for immunohistochemistry analysis of tyrosine hydroxylase (TH) and growth-associated protein 43 (GAP43).

Study protocol 2: LL-CBS on electrically or mechanically induced AF with acetylcholine

Group 2: In subgroup 1 (n = 13), to induce paroxysmal AF that closely resembles the paroxysmal AF observed in patients initiated by rapid focal firing from the PV-atrial junctions, we injected acetylcholine (Ach; 10 mM, 0.5 mL) into the anterior right ganglionated plexus (ARGP) as previously described.[9] In subgroup 2 (n = 11), applying Ach onto the atrial appendage induces sustained AF that shows focal characteristics arising from non-PV sites.[10] Three hours of LL-CBS was applied in the LL-CBS group (n = 7 and n = 6 in subgroups 1 and 2), while sham operation was performed in the control group (n = 6 and n = 5 in subgroups 1 and 2) (Figures 1H and 1I). The time duration of AF and the average AF cycle length were analyzed.

Statistical analysis

Data are presented as mean ± SEM. In group 1, paired *t* tests were used for comparisons within the LL-CBS group or control group. A multivariate analysis of variance was used for comparisons between the LL-CBS group and the control group. In group 2, an analysis of variance for repeated measures was used to compare changes before and after 3-hour LL-CBS.

Results

The detailed results are described in the Online Supplement.

During LL-CBS, BP and heart rate were stably maintained at the same level as baseline (Table 1). The average CBS threshold that induced a BP reduction of >10% was 2.5 ± 0.3 V (n = 21) without any change during 3-hour LL-CBS. The intensity of LL-CBS, which was 80% of the threshold, was 2.0 ± 0.2 V (n = 21).

ERP

In both LL-CBS and control groups, ERPs of all sites were markedly shortened by RAP in the first 2 or 3 hours (Figure 2). From the third hour on, ERPs in the control group were kept stable at an obviously lower level than at baseline. While in the LL-CBS group, compared to the level at the 3rd hour, ERPs were remarkably prolonged after initiating LL-CBS.