Time-dependent cervical vagus nerve stimulation and frequency-dependent right atrial pacing mediates induction of atrial fibrillation

Mingliang Rao, *Jialu Hu[1],* *Yan Zhang[2],* *Feng Gao[3],* *Feng Zhang[4],* *Zhi Yang[4],*
Xiaoya Zhang, *Yuemei Hou*

Department of Cardiology, Affiliated Fengxian Hospital, Southern Medical University
(Shanghai Fengxian Central Hospital); Shanghai-*China*
[1]Department of Cardiology, Zhongshan Hospital Affiliated to Fudan University; Shanghai-*China*
[2]Department of Cardiology, First Hospital of Fuzhou City; Fujian-*China*
[3]Department of Cardiology, Zhongshan Hospital Affiliated to Xiamen University; Fujian-*China*
[4]Department of Cardiology, The First Affiliated Hospital of Xinjiang Medical University; Xinjiang-*China*

Abstract

Objective: This study aimed to investigate the effects of right cervical vagus trunk simulation (RVTS) and/or right atrial pacing (RAP) on the induction of atrial fibrillation (AF).

Methods: Twenty-four healthy adult dogs were randomly divided into four groups: RAP groups comprising RAP_{500} (RAP with 500 beats/min) and RAP_{1000} (RAP with 1000 beats/min) and RVTS groups comprising RVTS and RAP_{500}+RVTS. All dogs underwent 12-h intermittent RAP and/or RVTS once every 2 h. The AF induction rate, AF duration, atrial effective refractory period (ERP), and dispersion of ERP (dERP) were compared after every 2 h of RAP or/and RVTS.

Results: All groups had successful AF induction. The RAP_{1000} group had the highest AF induction rate and the longest AF duration. The RAP_{1000} group also had a shortened ERP in comparison to the other groups as well as the maximum dERP. Compared to the RAP_{500} group, RAP_{500}+RVTS had an increased capacity to induce AF as measured by the AF induction rates, AF duration, ERP, and dERP.

Conclusion: Increased tension in the vagus nerve and the intrinsic cardiac autonomic nervous system plays an important role in AF induction through different potential mechanisms. Interventions involving the vagus nerve and/or intrinsic cardiac autonomic nervous system can be a future potential therapy for AF. *(Anatol J Cardiol 2018; 20: 206-12)*

Keywords: vagus trunk, right atrial pacing, atrial fibrillation, intrinsic cardiac autonomic nervous system

Introduction

Atrial fibrillation (AF) is a common multifaceted tachyarrhythmia causing an increased rate of morbidity, disability (1), and mortality in affected patients (1, 2). For many years, the prevailing mechanism for AF has been considered to be "multiple reentrant circuits," which is supported by computer modeling by Moe et al. (3) and involves the surgical maze procedure. Subsequently, research in the late 1990s demonstrated that pulmonary veins (PVs) are the most common trigger site for AF (4), which resulted in PV isolation (PVI) via radiofrequency (RF) ablation to become a gold standard treatment for paroxysmal AF (5).

Clinical and nonclinical studies revealed that the autonomic nervous system (ANS) is also an imperative component in AF initiation and progression. Lemola et al. (6) demonstrated that intact PVs are not required for the maintenance of experimental vagal AF and ganglionated plexi ablation may suppress the vagal response and prevent AF, indicating the importance of the ANS in the pathogenesis of AF. Although the association between cardiac innervation of the ANS from the brain and AF induction was well established during the last century (7), a majority of recent studies have focused on the roles of the ANS in terms of the mechanism (8-13) and/or treatment of AF (7, 14-18). Many theories have been proposed to explain the roles of the cardiac autonomic nervous system (CANS) in arrhythmia initiation and progression such as

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"The Third Fat Pad," (8) "Integration Center," (19, 20) "Octopus" hypothesis, (9) "Little brain," (10) and "Autonomic remodeling" (12); however, the roles of the CANS, including its upstream regulation during AF induction, remain inconclusive.

In the present study, we tested our hypothesis that increased tension in either the vagus trunk or the intrinsic CANS plays an important role in AF induction using a canine model. Our findings shed a light on future intervention involving the extrinsic CANS and/or intrinsic CANS in AF therapy.

Methods

Ethical consideration

The procedures involving animals were reviewed, approved, and supervised by the Ethics Committee of our institute.

Animal procedures

Twenty-four dogs were randomly divided into four groups: rapid right atrial pacing (RAP) groups comprising RAP_{500} (RAP with 500 beats/min of stimulation) (n=6) and RAP_{1000} (RAP with 1000 beats/min of stimulation) (n=6) and the right cervical vagus trunk stimulation (RVTS) groups comprising RVTS (n=6) and $RVTS+RAP_{500}$ (n=6). All animal studies were reviewed and performed in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (21). All 24 healthy adult dogs weighing 15–20 kg were anesthetized with 20 mg/kg sodium pentobarbital by intraperitoneal injection and ventilated with room air by a positive-pressure respirator (Hallowell EMC, Pittsfield, MA, US); the dogs also received additional doses (50–60 mg) administered hourly to maintain an adequate level of anesthesia. The core body temperature was maintained at 36.5±1.5 °C. The his bundle electrogram was recorded from a quadripolar electrode catheter introduced via the femoral artery and positioned in the aortic root. The blood pressure and standard lead II electrocardiogram were continuously monitored.

The thoracic cavity was accessed via a two-sided thoracotomy at the fourth intercostal space (22). The base of the left superior pulmonary vein (LSPV) and left inferior pulmonary vein (LIPV) were dissected from the visceral pleura, and a multi-electrode catheter was sutured to the visceral pleura in order to record or pace PVs. Similar electrode catheters were sutured to the left and right atria to record atrial electrograms and perform RAP. All recordings were displayed on a computer-based Lab System (LEAD-7000, Sichuan Jinjiang Electronic Science and Technology Co., Ltd., Chengdu, China) filtered at 30–350 Hz.

RVTS+RAP

Both cervical vagus trunks were dissected, and the location of the vagus trunk was verified via the vagal effect. A 0.1-mm silver wire was inserted into the right cervical vagus trunk (RVT). RVTS was performed by applying high frequency electrical stimulation (square waves, 1–8 V, 20 Hz, 0.5 ms) to the RVT via a Grass-S88

stimulator (Astro-Med Inc., West Warwick, RI, USA) (16). Before every 2-h stimulus, the threshold of RVTS was determined to adjust the voltage for RVTS for the next 2 h. RAP_{500} (1–40 V) was performed using the computer-based Lab System and RAP_{1000} (1–40 V) using a cardiac electrophysiology stimulator (DF-5A, Dongfang Inc., China). Each group underwent 12 h of stimulation intermittently.

Electrophysiological study

AF induction and the effective refractory period (ERP) in multiple sites (atria and PVs) were detected at baseline, prior to the various stimulations. Programmed stimulation at the right atrium (RA) was performed using a programmable cardiac stimulator (LEAD-7000 EP CONTROL, Sichuan Jinjiang Electronic Science and Technology Co., Ltd.), and the stimulation was suspended every 2 h to measure AF induction and ERP in different sites. In 18 dogs, atrial pacing at a cycle length of 300 ms (2×diastolic threshold) was performed at the multi-electrode catheter in the RA. Stimulation with progressively higher intensities was applied at the RVT until AF was induced; no AF was induced at 8 V in six dogs. AF was defined as irregular atrial rates faster than 500 beats/min with a duration of >5 s, associated with irregular atrioventricular conduction (9). Speed suppression or electrical cardioversion was used to terminate AF if the duration lasted >10 min. AF was induced five times with burst pacing in the atria or PVs to calculate the AF induction rate in the individual sites (S1-
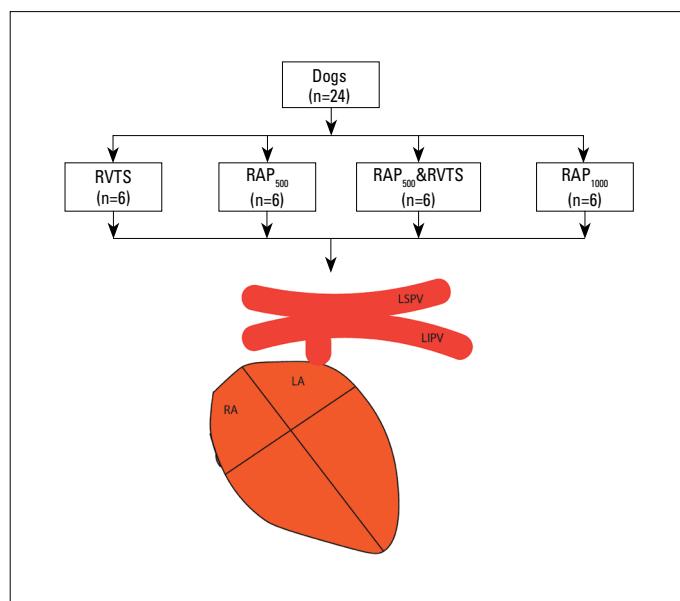
Figure 1. Experimental study design. Atrial fibrillation (AF) was induced in dogs under systemic anesthesia as described in the Methods section. A total of 24 dogs were included in this study and were randomly categorized into four groups: the right cervical vagus trunk stimulation (RVTS) group, the rapid right atrial pacing group stimulated with a frequency of 500 beats/min (RAP_{500}), the RAP_{500}+RVTS group, and the rapid right atrial pacing group stimulated with a frequency of 1000 beats/min (RAP_{1000}). Cardiac electrophysiological activities were recorded at the following sites—the left atrium (LA), the right atrium (RA), the left superior pulmonary vein (LSPV), and the left inferior vein (LIPV)

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S1 stimulation, 100 ms in cycle length, 2 ms in duration, and four-fold threshold current), which was defined as the relative ratio of the number of successful AF induction events to the total number of stimulations, expressed in percentage. The S1-S2 interval decreased from 200 ms to refractoriness initially in decrements (S1:S2=8:1, 1–40 V, 0.5 ms in duration). Moreover, dERP was calculated as the coefficient of variation [standard deviation (SD)/mean] of the ERP at all recording sites (Fig. 1) (23).

Statistical analyses

All data were expressed as mean±SD (24). The mean values of parameters in multiple groups were compared using two-way analysis of variance with Tukey post-hoc tests. A p value of <0.05 was considered statistically significant. All analyses were conducted using GraphPad Prism 7.0a (Mac Edition, GraphPad Software Inc, La Jolla, CA, USA).

Results

The RAP$_{1000}$ group shows increased AF induction

AF was induced with the various methods described above. The mean AF induction rate in each group at every time point was calculated (n=6). As shown in Figure 2 and Table 1, four methods used in this study were effective in inducing AF over time measured at four different anatomic sites–the LA, RA, LSPV, and LIPV. The RAP$_{1000}$ group had the highest AF induction rate compared with that of the RAP$_{500}$ (p<0.001), RVTS (p<0.0001), and RAP$_{500}$+RVTS (p < 0.05) groups at various sites (Fig. 2a-2d).

Moreover, the RAP$_{500}$+RVTS group had a higher induction rate compared with that of the RAP$_{500}$ group (p<0.05 or p<0.01 at different recording sites). These data indicate that the RAP$_{1000}$ group had the most effective influence on AF induction, and the RAP$_{500}$+RVTS group had a relatively strong effect on AF induction, suggesting that vagus stimulation and intrinsic CANS activation likely play a synergistic role in the pathogenesis of AF.
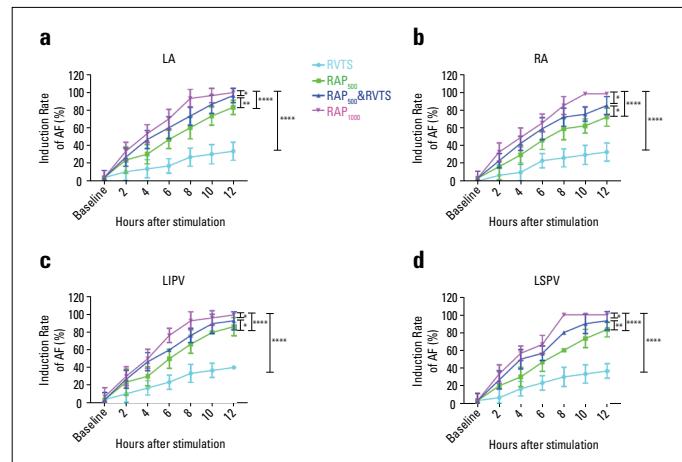
Figure 2. The RAP$_{1000}$ group shows increased AF induction rate. AF was induced and recorded at the following positions–the left atrium (LA, Panel A), the right atrium (RA, Panel B), the left inferior pulmonary vein (LIPV, Panel C), and the left superior pulmonary vein (LSPV, Panel D). The RAP$_{1000}$ group showed the highest AF induction rate in comparison to other groups. Meanwhile, the RAP$_{500}$+RVTS group had a higher AF induction rate compared with that of the RAP500 group. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001, analyzed by two-way analysis of variance with Tukey post-hoc tests (n=6)

Table 1. AF induction rates at recording sites in groups at 12 h after the initial stimulation

	RVTS	RAP$_{500}$	RAP$_{500}$&RVTS	RAP$_{1000}$	*P* value
LA	33.33±10.33	83.33±8.165	96.67±8.165	100±0	<0.0001
RA	33.33±10.33	73.33±10.33	86.67±10.33	100±0	<0.0001
LIPV	40±0	86.67±10.33	93.33±10.33	100±0	<0.0001
LSPV	36.67±8.165	83.33±8.165	93.33±10.33	100±0	<0.0001

LA - left atrium; RA - right atrium; LIPV - left inferior pulmonary vein; LSPV - left superior pulmonary vein; RVTS - right cervical vagus trunk stimulation; RAP - rapid atrial pacing; RAP$_{500}$ - RAP with a frequency of 500 beats/min; RAP$_{1000}$ - RAP with a frequency of 1000 beats/min. Data are presented as mean±standard deviation (SD). Statistical analyses were performed by two-way analysis of variance

Table 2. AF duration (s) at recording sites in groups at 12 h after the initial stimulation

	RVTS	RAP$_{500}$	RAP$_{500}$&RVTS	RAP$_{1000}$	*P* value
LA	13.17±3.601	26±4.427	34.17±3.764	45.81±4.011	<0.0001
RA	13.67±4.179	27.17±4.75	35.83±2.317	44.5±1.871	<0.0001
LIPV	16.83±2.317	30.83±0.9832	38.67±1.751	48.17±0.4082	<0.0001
LSPV	16±3.406	29.83±0.7528	36.83±1.722	46.83±0.7528	<0.0001

LA - left atrium; RA - right atrium; LIPV - left inferior pulmonary vein; LSPV - left superior pulmonary vein; RVTS - right cervical vagus trunk stimulation; RAP - rapid atrial pacing; RAP$_{500}$ - RAP with a frequency of 500 beats/min; RAP$_{1000}$ - RAP with a frequency of 1000 beats/min. Data are presented as mean±standard deviation (SD). Statistical analyses were performed by two-way analysis of variance

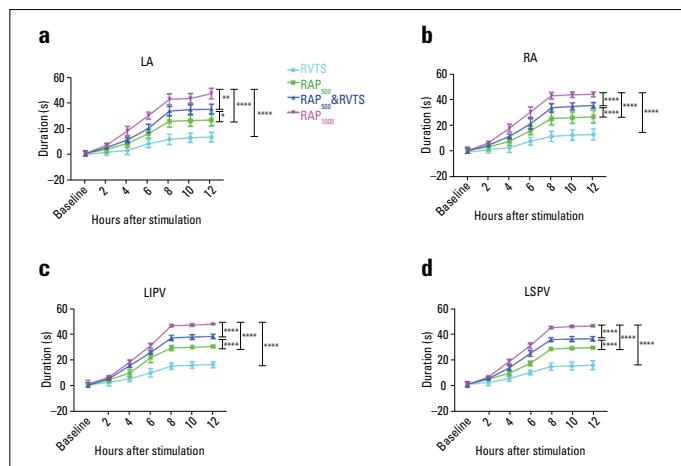Anatol J Cardiol 2018; 20: 206-12
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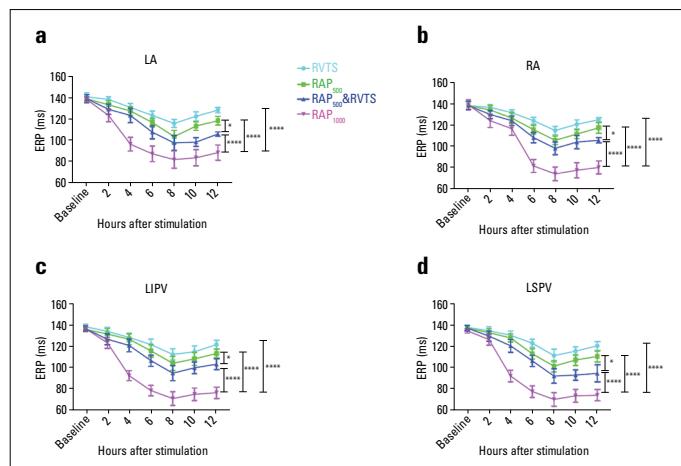**The RAP$_{1000}$ group has longer AF duration at all recording sites**

AF duration was also recorded in various groups at the same four anatomic sites. As illustrated in Figure 3 and Table 2, the RAP$_{1000}$ group showed a significantly longer AF duration recorded at all four sites in comparison to the RVTS, RAP$_{500}$, and RAP$_{500}$+RVTS groups. It is worth noting that the RAP$_{500}$+RVTS group had longer AF durations in comparison to the RAP$_{500}$ group (p<0.05, p<0.01, or p<0.0001, according to different recording sites).

The RAP$_{1000}$ group has a shortened ERP

A shortened ERP is positively correlated with increased susceptibility of AF (25), and medications terminate AF by prolonging ERP (26); therefore, we investigated the effects of different stimulations on ERPs at various time points at the four anatomic sites. As shown in Figure 4 and Table 3, the RAP$_{1000}$ group showed the shortest ERP at all recording sites, highlighting the effects of atrial pacing and RVTS in shortening ERPs. Moreover, the RAP$_{500}$+RVTS group had a shorter ERP compared with that of the RAP$_{500}$ group (p<0.05 at all recording sites). We also observed

Figure 3. The RAP$_{1000}$ group shows increased AF duration. AF was induced and recorded at the following positions–the left atrium (LA, Panel A), the right atrium (RA, Panel B), the left inferior pulmonary vein (LIPV, Panel C), and the left superior pulmonary vein (LSPV, Panel D). The RAP$_{1000}$ group had a longer AF duration compared with that of other groups. Meanwhile, the RAP$_{500}$+RVTS group had longer AF duration in comparison to the RAP500 group. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001, analyzed by two-way analysis of variance with Tukey post-hoc tests (n=6)

Figure 4. The RAP$_{1000}$ group shows significantly shortened effective refractory period (ERP). AF was induced and recorded at the following positions–the left atrium (LA, Panel A), the right atrium (RA, Panel B), the left inferior pulmonary vein (LIPV, Panel C), and the left superior pulmonary vein (LSPV, Panel D). The RAP$_{1000}$ group had a significantly shorter ERP compared with that of other groups. The RAP$_{500}$+RVTS group had a shorter ERP compared with that of the RAP$_{500}$ group. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001, analyzed by two-way analysis of variance with Tukey post-hoc tests (n=6)

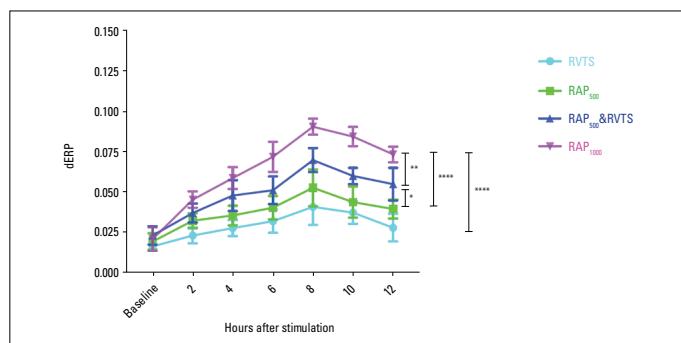**Table 3. AF ERP (ms) at recording sites in groups at 12 h after the initial stimulation**

	RVTS	RAP$_{500}$	RAP$_{500}$&RVTS	RAP$_{1000}$	*P* value
LA	128.3±2.582	118.3±4.082	105.8±2.041	88.33±7.146	<0.0001
RA	125.8±2.041	118.3±5.164	106.7±2.582	81.33±6.022	<0.0001
LIPV	121.7±4.082	113.3±4.082	103.3±5.164	76.67±5.317	<0.0001
LSPV	121.7±4.082	111.7±5.164	95.83±8.01	75.33±5.317	<0.0001

ERP - effective refractory period; LA - left atrium; RA - right atrium; LIPV - left inferior pulmonary vein; LSPV - left superior pulmonary vein; RVTS - right cervical vagus trunk stimulation; RAP - rapid atrial pacing; RAP$_{500}$ - RAP with a frequency of 500 beats/min; RAP$_{1000}$ - RAP with a frequency of 1000 beats/min. Data are presented as mean±standard deviation (SD). Statistical analyses were performed by two-way analysis of variance

Table 4. AF dERP at 12 h after the initial stimulation

RVTS	RAP$_{500}$	RAP$_{500}$&RVTS	RAP$_{1000}$	*P* value
0.0285±0.008	0.04±0.005	0.055±0.010	0.0735±0.004	<0.0001

dERP - dispersion of effective refractory period; LA - left atrium; RA - right atrium; LIPV - left inferior pulmonary vein; LSPV - left superior pulmonary vein; RVTS - right cervical vagus trunk stimulation; RAP - rapid atrial pacing; RAP$_{500}$ - RAP with a frequency of 500 beats/min; RAP$_{1000}$ - RAP with a frequency of 1000 beats/min. Data are presented as mean ± standard deviation (SD). Statistical analyses were performed by two-way analysis of variance

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Figure 5. The RAP_{1000} group shows the most prolonged dispersion of effective refractory period (dERP). AF induction and dERP measurement are described in the Methods section. The RAP_{1000} group had the most prolonged dERP. *, $p<0.05$; **, $p<0.01$; ****, $p<0.0001$ analyzed by two-way analysis of variance with Tukey post-hoc tests (n=6)

that the effects of stimuli in shortening ERPs were not significant after 8 h (analyses not shown), indicating the importance of stimuli during the initial phases of AF.

The RAP_{1000} group has increased dERP

Increased dERP has been reported to be well correlated with vulnerability of AF (24); therefore, we investigated the consequences of stimulation in dERP. As shown in Figure 5 and Table 4, the RAP_{1000} group had the maximum dEFP compared with that of the other groups, indicating the importance of rapid atrial stimulation in the pathogenesis of AF. The RAP_{500}+RVTS group had a higher dERP compared with that of the RAP_{500} group. It is worth noting that the peak effects in increasing dERP occurred at 8 h following the initial stimulation, and further effects were observed after the 8-h time point in all four groups (statistical analysis not shown), suggesting the significance of stimulation to the RA and RVT during the initial phases of AF.

Discussion

In the present study, we investigated the significance of stimulation to the RA and RVT in the pathogenesis of AF. Our findings indicated that (1) the RAP_{1000} group had the highest AF induction and longest AF duration, (2) the rapid right atrial stimulation (the RAP_{1000} group) and invigoration in the RA and RVT (RAP_{500}+RVTS) shortened the ERP, and (3) the RAP_{1000} group had the most pronounced increase in dERP during the initial phases of AF. These data indicate a possible mechanism of RAP and/or RVTS in AF induction—RAP and/or RVTS mediates AF induction by decreasing the ERP during the entire event and increasing dERP during the initial phases.

ANS has been shown to have an important role in the initiation and maintenance of AF (17). Although previous studies have reported that sympathetic stimulation may be a trigger for AF (27), increasing evidence has indicated that the parasympathetic nervous system also has a significant role in initiating AF [reviewed in (28)]. Activation of either the extrinsic parasympa-

thetic or sympathetic neural elements of the CANS has been reported to induce rapid focal firing and AF induction via mechanisms of shortening the atrial or PV refractoriness mediated by parasympathetic neurotransmitters or increasing intracellular Ca^{2+} concentrations mediated by sympathetic neurotransmitters, respectively. These results indicate the complexity and multiple functions of the CANS in inducing AF (29). A recent study also indicated that the autonomic imbalance between the sympathetic and parasympathetic tensions could be either pro-arrhythmic or anti-arrhythmic (30). In the present study, we utilized RVTS to successfully induce AF by reducing the ERP and increasing dERP, which was in agreement with published reports (31-34). Although the underlying mechanisms of RVTS-induced AF remain elusive, we hypothesized that RVTS activates the extrinsic and/or intrinsic CANS to mediate neural remodeling, which initiates and/or maintains AF presumably by increasing the activity of acetylcholine. Further investigations are warranted for investigating these mechanisms.

Previous studies have also reported that direct or indirect stimulation of the vagus nerve was able to inhibit established AF by various mechanisms (35-39). It is worth noting that those aforementioned studies were performed using different methods to stimulate the vagus nerve-either with a lower intensity or by percutaneous stimulation. In addition, the afferent input from vagus nerve stimulation to the central nervous system has yet to be excluded. All these considerations may explain why we induced AF rather than suppressed AF by RVTS under our experimental conditions and highlight the complexity of the ANS and CANS. In our study, we also observed that the RAP_{500}+RVTS group had increased efficacy to induce AF in comparison to the RAP_{500} group, suggesting that stimulation of the parasympathetic nervous system has a synergistic role with stimulation of atrial cardiomyocytes during AF initiation.

With respect to a previous publication (36), atrial pacing induced AF similar to the RAP_{500} and RAP_{1000} groups in our study. Based on the different frequencies, stimulation in the atrium might have different roles. Considering the duration of the ERP in atrial cardiomyocytes, most stimuli of RAP_{500} may fall outside the ERP duration, thus RAP_{500} likely activates atrial cardiomyocytes to induce AF. Alternatively, most stimuli of RAP_{1000} may fall within the ERP duration, and therefore, RAP_{1000} could mediate neural remolding to stimulate the intrinsic CANS and trigger AF. However, further studies are required to assess the potential association between the frequencies of pacing and the targets of pacing.

Clinical relevance

In the current study, we demonstrated that RVTS induced AF, which had a synergistic effect when paired with atrial pacing; various frequencies in atrial pacing had different possible mechanisms to induce AF. These results suggest that physicians should measure the tone of the vagus nerve to distinguish AF with a normal vagus tone from those with a higher tension in order to establish a personalized therapeutic strategy. As the

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stimulation of RAP_{1000} may cause neural remodeling, AF with rapid atrial rates may require intervention of the intrinsic CANS.

Study limitations

The current study has certain potential pitfalls. First, we used short-term RAP to induce AF rather than chronic RAP, because the major objective of this study was to determine the effects of RVTs. Second, we did not consider the effects of sympathetic nerve stimulation. Lastly, we did not compare the current experimental system with established low-level vagosympathetic nerve stimulation and low-level transcutaneous stimulation models. Because this pilot study indicated the significance of the clinical application and future potential therapies for AF, we will conduct additional investigations based on these current results.

Conclusion

In conclusion, a high-tension state of the vagus trunk initiates AF by affecting the activity of the intrinsic CANS or by promoting acute electrical remodeling during short-term RAP, which is helpful in AF initiation and progression during the initial phase. In addition, the activation of the intrinsic CANS may enhance the acute electrical remodeling that depends on or cooperates with extrinsic CANS activity during RAP.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – Y.H.; Design – M.R., Y.H.; Supervision – M.R., F.G., Y.H.; Fundings – Y.Z., F.G., Y.H.; Materials – Y.Z., F.G.; Data collection &/or processing – Y.Z., F.Z., Z.Y., X.Z.; Analysis &/or interpretation – M.R., J.H., Y.H.; Literature search – M.R., J.H.; Writing – M.R., J.H.; Critical review – M.R., J.H., Y.H.

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