Cellular and Molecular Biology

Zhang *et al. Cell. Mol. Biol.*2016, 62 (7): 80-84
ISSN: 1165-158X
doi: 10.14715/cmb/2016.62.7.14

Original Research

Norepinephrine and acetylcholine changes during electrically-induced atrial fibrillation episodes in canine models

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Abstract: Atrial fibrillation (AF) is the most prevalent heart rhythm disorder, and autonomic nervous system (ANS) is important to AF. This study aims to identify whether changes in transmitters released by ANS could reflect their activities. The right atrium (RA) groups (1-40V) included RA_{500} and RA_{1000}. While ANS groups received high-frequency electrical stimulation (1-8V, 20 Hz, 2 ms), including left stellate ganglion stimulation (LSGS) andleft cervical vagus trunk stimulation (LVTS). The induced rate of AF, duration and atrial effective refractory period (AERP) were measured. The blood was drawn for evaluation of norepinephrine (NE) and acetylcholine (Ach) concentrations. At 12-hours, RA tissue was dissected and compared against un-stimulated controls. While AF was induced by all groups, duration and AERP were significantly different between RA pacing groups and ANS-stimulated groups, respectively ($P<0.05$). Specific changes in profile of NE and Ach were associated with modality of stimulation. RA_{1000} tended to display most significant changes ($P<0.05$) compared to other groups while variables concentration levels were observed in other groups. In conclusion, electrically-induced AF initiated by various modalities of stimulation showed different changes in serum and RA tissues. Fast frequency pacing caused significant atrial electrical remodeling, including ANS activity change.

Key words: Atrial fibrillation, electrically induced, zoopery, norepinephrine, acetylcholine.

Introduction

Atrial fibrillation (AF) is the most prevalent heart rhythm disorder, affecting more than 33 million people world-wide (1). AF is associated with increased risk of morbidity and mortality, stroke and worsening of heart failure (2). Electrical, structural and contractile remodeling represents important mechanisms leading to AF (3-5). Numerous studies have shown that the autonomic nervous system (ANS) plays an important role in AF (6-9). ANS directly influence AF, however the mechanisms through which this occurs remains incompletely characterized. Treatment for AF involves both pharmacological and surgical intervention including: β-receptor blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers and atrial ganglion plexus ablation. In recent years, researchers have measured ANS signals using remote wireless telemetry technology in animal models of AF. While the findings have been meritous, this technology is not currently suited for clinical applications. ANS signaling includes a balancing act of cues received from the sympathetic and parasympathetic nerves. Norepinephrine (NE) is released from the sympathetic nerves, stimulating heart rate and cardiac conduction, while acetylcholine (Ach) released from vagus nerve slows hearts rate and cardiac conduction (10). In the current study, we aimed to identify whether changes in transmitters released by ANS could reflect their activities, such as increased, decreased or stability during electrically-induced AF episodes. Our study involved the use of sustained ANS and right atrium (RA) stimulation for 12 hours followed by an analysis of electrophysiological evaluation of the RA, and an evaluation of the associated changes in serum and RA tissues concentrations of NE and Ach.

Materials and Methods

Animal preparation

Sixteen (n=16) healthy adult mongrel dogs (15-20 kg) were divided into 4 groups. These groups included: ① RA pacing with frequency 500bpm (RA_{500}, n=4) and ②RA pacing with frequency 1000bpm (RA_{1000}, n=4), ③ left stellate ganglion stimulation (LSGS, n=4) and ④ left cervical vagus trunk stimulation (LVTS, n=4). All dogs were anesthetized with Na-pentobarbital (20 mg/kg) (11). Additional doses of 30-40 mg were administered hourly to maintain an adequate level of anesthesia (12). Positive pressure ventilation was applied through an endotracheal tube. Blood pressure and electrophysiology were continuously measured using a Lead 7000 instrument (Jinjiang Inc., China).

RA and ANS stimulation

ANS stimulated groups were exposed to LSG and LVT. Silver electrodes were fixed on the LSG or LVT and a multielectrode catheter was sent into each canine RA via external jugular vein (11). All tracings from the electrode catheters were amplified and recorded using a computer-based Lab System (Lead 7000, Jinjiang Inc., China).

Stimulation of LSG and LVT was performed by applying high frequency electrical stimulation (square

Received February 18, 2016; **Accepted** June 14, 2016; **Published** June 30, 2016

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waves, 1-8 V, 20 Hz, 0.5 ms) to the LSG and LVT via a Grass-S88 stimulator (Astro-Med Inc., USA) (13). RA_{500} stimulated by Lead 7000, RA_{1000} stimulated by a cardiac electrophysiology stimulator (DF-5A, Dongfang Inc., China), the two groups stimulated by 1-40V. For each group, stimulation was applied for 12 hours.

Electrophysiological studies

Stimulation was arrested every 2 hours, ten minutes later, AF was induced 3 times with RA burst pacing by 30 seconds (500 bpm, 1-40 V) within 30 minutes, calculated AF-induced rate and duration. AF was defined as irregular atrial rates>500 beats/min and a duration>5 seconds, AF>30 minutes, gave speeding suppression or electrical cardioversion to terminate (11,12). Atrial effective refractory period (AERP) was measured at an atrial pacing cycle length of 300 ms, the S1-S2 intervals decreased from 200 ms to refraceoriness initially in decrements of 5 ms (S1:S2=8:1, 1-40V, 0.5 ms in duration), AERP was defined as the longest S1-S2 interval failing to produce a response (11,12). AF induction rate was defined as the relative ratio of successful induction frequency / total frequency of stimulation 100%.

Evaluation of serum and RA tissues

Baseline blood (5 mL) was obtained before stimulation. During stimulation blood was also collected at four hour intervals. The serum was stored at -80°C. Twelve hours following the experiment the canine heart was dissected and kept at -80°C. NE and Ach concentrations in the serum were evaluated against baseline measurements, while these change within RA tissues were compared against unstimulation tissues (control group, n=4).

NE and Ach serum levels and RA tissue concentrations were tested by OD value using an ELISA kit (Force sensitive, Inc., China.).

Statistical analysis

Data were analyzed by SPSS20.0 software, with data expressed as means±standard deviation (SD). Statistical comparisons for data between groups was performed using analysis of variance (ANOVA) or nonparametric tests (two or more independent samples). Multiple-group repeated measurement comparisons were performed using analysis of general linear model repeated measures. Values below a statistical significance of P<0.05 were considered statistically significant.

Results

Electrophysiological analysis

Atrial fibrillation of each group was induced. Compared to baseline levels, all an AF-induced increased rate of burst pacing following stimulation (P<0.05). There were no significant differences observed between RA_{500} and RA_{1000} (P>0.05). The AF-induced rate of LSGS was similar to LVTS (P>0.05). Notably, in the ANS stimulation group, the induction rate continued to increase over the entire stimulation period whereas a plateau was observed for RA groups after 8-hours of stimulation (Figure 1).

Moreover, the duration of AF following stimulation was also significantly increased (P<0.05) across

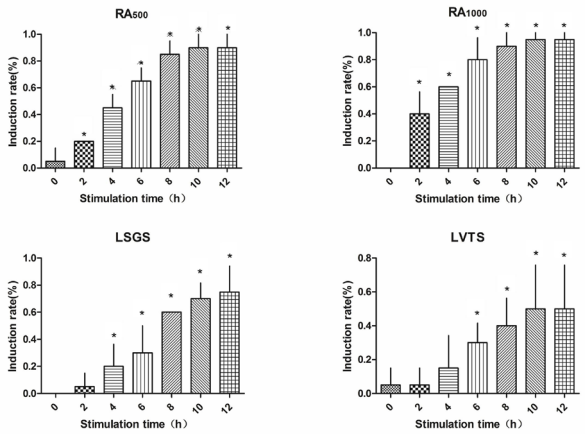

Figure 1. The atrial fibrillation induced rate of each group. * P<0.05 compared with baseline of each group. LSGS: Left stellate ganglion stimulation. LVTS: Left cervical vagus trunks stimulation. RA_{500}: Right atrium pacing with frequency 500bpm. RA_{1000}: Right atrium pacing with frequency 1000bpm.

all groups as compared to baseline measurements. The AF duration at RA_{1000} was longer than RA_{500} (P<0.05). There were no significant differences observed between LSGS and LVTS (P>0.05). Following 8 hours stimulation, there was no further significant changes were observed in the duration time for each group (Figure 2).

Compared to baseline, the AERP shortened upon stimulated (P<0.05), there were significant difference between RA_{500} and RA_{1000} (112.321±15.244 ms vs 102.500±18.930 ms, P<0.05). However, there were not difference between LSGS and LVTS groups, respectively (127.679±11.665 ms vs 129.107±14.533 ms, P>0.05). AERP was significantly shortened by atrial pacing (P<0.05) (Table 1).

Serum levels of NE and Ach

When compared against baseline levels modulations in NE were observed over the course of a 12-hour stimulation. In the RA_{500} group, the level of NE was lowest at 4-hours (147.692±30.533 pg/ml, P<0.05), and highest at 8-hours (195.722±30.831 pg/ml, P<0.05). In

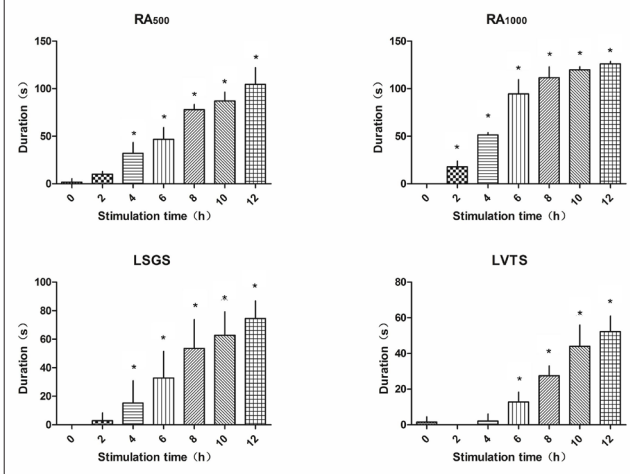

Figure 2. The atrial fibrillation duration of each group. * P<0.05 compared with baseline of each group. LSGS: Left stellate ganglion stimulation. LVTS: Left cervical vagus trunks stimulation. RA_{500}: Right atrium pacing with frequency 500bpm. RA_{1000}: Right atrium pacing with frequency 1000bpm.

Table 1. The atrial effective refractory period shortened of each group.

Group	0-hour	2-hour	4-hour	6-hour	8-hour	10-hour	12-hour
RA_{500}	137.500±3.227	126.250±3.146*	115.000±2.041*	106.250±3.146*	93.750±2.394*	98.750±2.394*	108.750±2.394*
RA_{1000}	136.250±1.250	117.50±3.227*	96.250±1.250*	81.250±1.250*	83.750±3.146*	93.750±1.250*	108.75±2.394*
LSGS	143.750±3.146	142.500±1.443	128.750±1.250*	120.000±3.536*	113.750±2.394*	122.500±1.443*	122.500±3.227*
LVTS	146.250±6.250	136.250±7.465*	127.500±8.292*	123.750±6.250*	117.500±5.204*	123.750±6.250*	128.750±5.154*

*P<0.05 compared with baseline of each group. LSGS: Left stellate ganglion stimulation. LVTS: Left cervical vagus trunks stimulation. RA_{500}: Right atrium pacing with frequency 500bpm. RA_{1000}: Right atrium pacing with frequency 1000bpm.

contrast, peak levels of NE within the RA_{1000} group were at 4-hour (218.338±13.850 pg/ml, P<0.05), while LSGS and LVTS maximums were observed at 4-hours respectively (LSGS: 248.273±20.675 pg/ml, P<0.05; LVTS 247.713±13.760 pg/ml, P<0.05). The lowest observed values for NE occurred at 4-hours for the RA_{500} group. Interestingly, this is the same time the other groups achieved their maximum values (Figure 3).

Similar analysis was pursued for Ach and summarized in Figure 3. The lowest levels of Ach were observed within the RA_{500} group at 4-hours (50.525±4.533 pmol/ml, P<0.05). RA_{1000}, LSGS and LVTS all achieve peak measurements at 4-hours (RA_{1000}: 78.213±4.017 pmol/ml, P<0.05; LSGS 62.218±4.581 pmol/ml, P<0.05; LVTS: 75.197±4.792 pmol/ml, P<0.05). The lowest levels of RA_{500} were observed at 4-hours of stimulation (Figure 4).

NE and Ach concentrations of RA tissues

Comparison of control group (632.487±11.743 pg/ml), NE concentration of RA_{1000} (428.627±19.214 pg/ml) and LSGS (488.591±8.288 pg/ml) both were reduced following stimulation (P<0.05). NE and Ach concentrations were consistency lowest in the RA_{1000} group while the LVTS group demonstrated the highest concentration values observed (Figure 5).

Discussion

In this study, we established an *in vivo* canine models of electrically-induced AF and demonstrated that: ① AF induction rate, duration and AERP shortened of atrial pacing groups were higher than ANS groups (P<0.05). AF induction rate and duration increased with

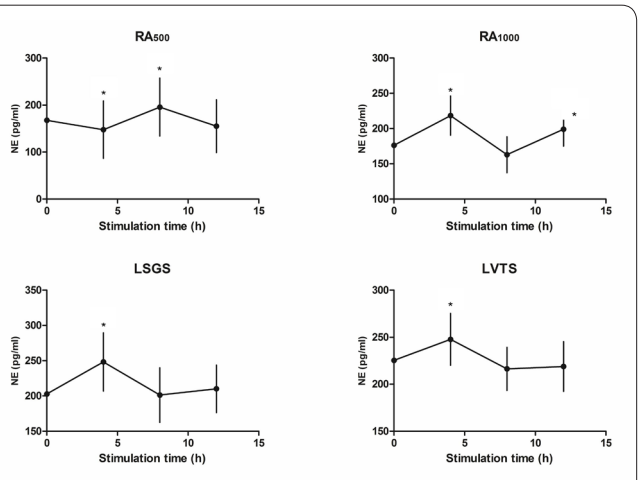

Figure 3. Norepinephrine serum levels of each group. * P<0.05 compared with baseline of each group. LSGS: Left stellate ganglion stimulation. LVTS: Left cervical vagus trunks stimulation. RA_{500}: Right atrium pacing with frequency 500bpm. RA_{1000}: Right atrium pacing with frequency 1000bpm.

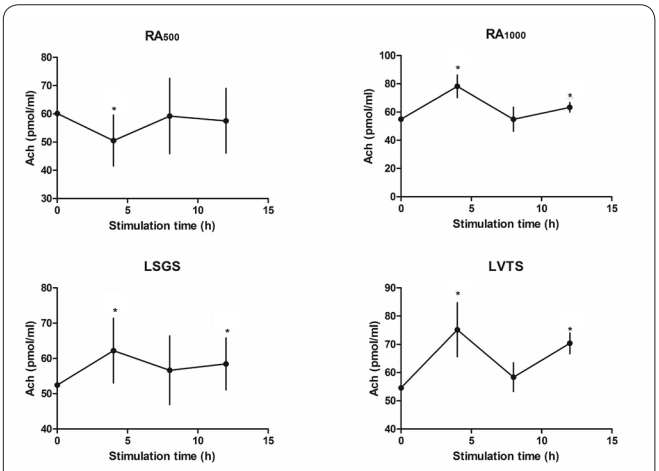

Figure 4. Acetylcholine serum levels of each group. * P<0.05 compared with baseline of each group. LSGS: Left stellate ganglion stimulation. LVTS: Left cervical vagus trunks stimulation. RA_{500}: Right atrium pacing with frequency 500bpm. RA_{1000}: Right atrium pacing with frequency 1000 bpm.

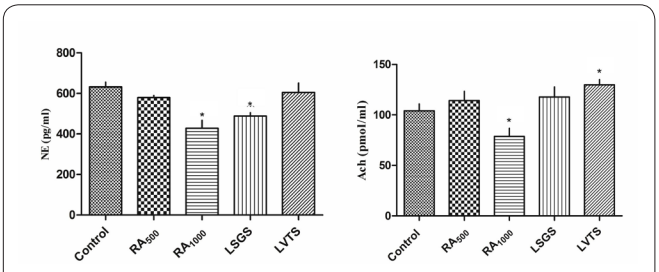

Figure 5. Norepinephrine and Acetylcholine concentrations of each group. * P<0.05 compared with control group. Control group: without any stimulation. LSGS: Left stellate ganglion stimulation. LVTS: Left cervical vagus trunks stimulation. RA_{500}: Right atrium pacing with frequency 500bpm. RA_{1000}: Right atrium pacing with frequency 1000 bpm.

for the first 8-hours of stimulation before normalizing to a steady state. ② Modulations in NE and Ach serum levels were readily detectable with lowest levels observed after 4-hours of pacing in the RA_{500} group, while, conversely, the highest levels were observed at this same time point for the RA_{1000}, LSGS and LVTS groups. ③ When compared against control group RA tissues, RA_{1000} and LSGS consumed more NE than other groups.

Since the last century, the vagus nerve had been used to induce and maintain AF (14), and direct autonomic nerve recordings in canine models have demonstrated that simultaneous sympathovagal discharges are the most common triggers of PAF (15). Our research demonstrated that pacing and ANS stimulation both triggered AF, and shorten the AERP. As it is known that catecholamines and Ach shorten the refractoriness in atrium (12,16). We measured the levels of NE and Ach

levels change reflected in peripheral blood and RA tissues following different methods of stimulation. However our results reflect that RA tissues change trend was not fully supported by changes in serum levels.

Activation of ANS by the central nervous system stimulates transmitter release, but NE and Ach release from cardiac nerves is regulated in the atria by activation of inhibitory presynaptic receptors (17,18). As such, serum levels and RA tissues contents matched imperfectly in our study.

Although we stimulated unilateral nerve, i.e., LVT or LSG, there was still some influences from right vagal nerve trunk, right stellate ganglion, peripheral nerve and central nervous system, and even non-neuronal cells. We considered, therefore, that the four groups all accept complex neurological cues, however different methodologies of stimulation result in downstream effects.

It has also been suggested that all groups undergo electrophysiology and ANS changes during the AF. In our study, the RA_{1000} group was most impacted. NE and Ach contents levels of serum showed a precise temporal changes. Neurotransmitters of RA_{1000} changed dramatically in both the serum and tissues, we hypothesize that pacing with fast frequency caused significant atrial electrical remodeling, including ANS activity change. Neurotransmitters of RA_{500} did not change like RA_{1000} or ANS groups, conforming the findings of Wijiffels et al. (3) (i.e. "AF begets AF"). LSGS and LVTS were mainly modulated by ANS in the serum. The results of RA tissues showed that local ANS has a great influence on myocardium, suggesting AF treatment is considerably more complex than originally believed. However, these changes caused by pure electrical stimulation or AF were still unclear. As such, we concluded that electrically-induced AF initiated by different stimulation method present diverse changes in serum and RA tissues, whether NE and Ach serum levels could be a expanded for the use in clinical diagnosis of AF should identified via clinic trial.

Though we have received a few interesting results, there were also some limitations of our study. Firstly, our samples size was small. This should be rectified in subsequent studies. Secondly, the serum levels were tested every 4-hour without continuous monitoring. As such, we do not know the change that occurs between each time points. Thirdly, we believe the the model we used is not a sufficiently pure to clearly elucidate the impact of specific modalities of stimulation. Fourthly, the canine's model is an experimental model, and atriovenous dysfunction contributes to the initiation of AF. Therefore, the application for human also needs some of the further exploration. We feel strongly that new models need to be created to expand and improve upon these findings

In conclusion, electrically-induced AF initiated by various modalities of stimulation showed different changes in serum and RA tissues. Fast frequency pacing caused significant atrial electrical remodeling, including ANS activity change.

Acknowledgements

The work was supported by grant from Shanghai Municipal Commission of Health and Family Planning of China (20134009, http://www.wsjsw.gov.cn/wsj/). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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