Atrial electrical remodeling in a canine model of sinus node dysfunction

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A R T I C L E   A B S T R A C T

Aims: To study atrial tachycardia-induced electrical remodeling in a canine model of sinus node dysfunction (SND).

Materials and methods: A canine model of SND was established by contacting a cotton patch with 20% formaldehyde on the sinus node. Atrial effective refractory period (ERP), ERP dispersion, and inducibility of atrial fibrillation (AF) were recorded at multiple sites in the atrium, before and after SND induction as well as after rapid atrial pacing. The recovery of atrial ERP in the left and right atrium (LA and RA) after cessation of atrial pacing was also recorded.

Results: Compared with baseline, the atrial ERPs were shortened after SND (P<0.05). After rapid atrial stimulation, the atrial ERPs were further decreased significantly (P<0.05), and the dispersion of atrial ERPs measured at different pacing cycle lengths (PCLs) showed significant variation. Seven sites were used to induce AF in each dog (56 sites in 8 dogs). The average duration and inducibility of AF after SND was increased compared with baseline (16.5±4.7 vs 2.3±1.2 s and 12/56 vs 4/56 sites, P<0.05). After rapid atrial stimulation, the average duration and inducibility of AF were further increased (16.5±4.7 vs 33.6±16.1 s and 12/56 vs 25/56 sites, P<0.05). The recovery of atrial ERP in LA was significantly delayed compared to the RA.

Conclusion: SND induces atrial electrical remodeling which is further aggravated by atrial tachycardia. Therefore, SND creates an electrophysiological substrate that facilitates AF initiation and perpetuation.

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1. Introduction

Atrial fibrillation (AF) is a heterogeneous condition with complex pathophysiology. Accumulating evidence suggests that, apart from the triggers, AF development and perpetuation depends on the electrical and structural remodeling of the atria [1]. Atrial electrical remodeling seems to promote AF and it has been associated with the early recurrences after cardioversion [1,2]. The principal characteristics of this process are the shortening of the atrial effective refractory period (ERP) with increased dispersion, the loss of its rate adaptation, and the reduction of atrial conductivity [1–3]. Interestingly, these changes can be reversed within a few days after cessation of atrial tachycardia, a process termed reverse atrial electrical remodeling [1–3]. The concept of tachycardia-induced atrial electrical remodeling was introduced in 1995 by 2 independent experimental studies [4,5].

On the other hand, brady–tachy syndrome represents a common clinical subtype of sick sinus syndrome (SSS), manifesting as periods of bradycardia and/or sinus arrest alternating with periods of atrial tachyarrhythmias (AF, atrial tachycardia, or atrial flutter) [6]. Recent data on the electrophysiological and electroanatomic characterization of the human atria in sinus node disease indicate diffuse atrial remodeling characterized by structural abnormalities, widespread conduction slowing, and increased right atrial refractoriness [7]. However, the atrial vulnerability to AF during sinus node disease has not been studied. Moreover, patients with SSS often have comorbidities which promote both structural and electrical remodeling. Thus, the potential effect of sinus node dysfunction (SND) on atrial electrophysiological properties cannot be easily studied.

In the present study, we sought to study the atrial electrical remodeling in a canine model of SND and to further examine the effect of this process on AF inducibility.

2. Materials and methods

The study was conducted after obtaining approval by the Experimental Animal Administration Committee of Tianjin Medical University and Tianjin Municipal Commission for Experimental Animal Control. Twelve Mongrel dogs (12–22 kg) were initially instrumented and anesthetized by 3% pentobarbital (30 mg/kg). Subsequently, tracheal intubation and respiration assistance was provided (KTH-2 Respirator, China). The aortic blood pressure was continuously monitored by carotid cannulation (Nihon Kohden VC-22) as well as the arterial blood gases by a blood gas system (Ciba-Conning 288, USA) in order to maintain blood pressure, PaO2, pH, and electrolytes within normal limits. Exposure of the heart was performed by mid-thoracotomy and creating an epicardial cradle.

In each open chest dog, six pairs of electrodes (diameter: 1.5 mm and distance between poles: 1.5 mm) were sewn on the left and right atrial (LA and RA) epicardium. The corresponding sites were at the high left and right atrium (HLA and HRA), mid left and right atrium (MLA and MRA), and low left and right atrium (LLA and LRA). Furthermore, a pair of electrodes (7th site) was positioned at the epicardium which was in proximity to Bachmann’s bundle. The locations of electrodes are depicted in Fig. 1.
the induced AF was persistent, atrial defibrillation was performed to restore sinus rhythm. In cases where AF could not be induced by rapid stimulation, the atrial stimulation was continued in order to complete a total duration of 2 h (inducible AF and 2-hour rapid atrial pacing were considered as a '2-hour stimulation'). HR and atrial ERP at PCL350, PCL250 and PCL200 were measured again immediately after the 2-hour period of stimulation, while the inducibility and duration of AF at the aforementioned sites was assessed.

2.3. Protocol of electrophysiological studies

Atrial ERP was evaluated using programmed extra-stimuli and defined as the longest S1–S2 interval that failed to capture. The S2 extra-stimulus was delivered after a drive-train of 8 S1 stimuli. The S1–S2 interval was decreasing in intervals of 2 ms until atrial refractoriness was reached. The mean of 3 atrial ERP values was used for data analysis. The dispersion of atrial ERPs was calculated as the maximal minus minimal value measured in the 7 different sites. AF induction was defined as P wave disappearance and rapid atrial activation with irregular ventricular response (longer than 1 s on atrial ECG) after programmed atrial stimulation (S1–S2). The site where AF could be induced was denoted as AF induction site. The duration of induced AF was also recorded. Furthermore, recovery of atrial ERPs at PCL350, PCL250 and PCL200 at the 7 sites was recorded 20 min, 40 min, 60 min, 80 min, 100 min, 120 min, and 140 min after the 2-hour stimulation period.

3. Statistical analysis

Continuous variables were expressed as means ± standard deviation (SD) and categorical variables as frequencies. Differences among multiple groups were examined by one way analysis of variance (ANOVA). Post hoc comparisons were performed with the Bonferroni test. A Chi-square test with Fisher's exact test was used for the categorical data. A value of P < 0.05 was considered to be statistically significant.

4. Results

Eight out of 12 dogs were successfully established SND and included in the experimental protocol and final data analysis. SCL prolongation occurred within 3 to 5 min and was stable 60 min after formaldehyde cotton pad application. After the 2-hour stimulation period 3 animals were on AF, and therefore atrial electrical defibrillation was performed in order to proceed to the electrophysiological studies.

4.1. Changes of heart rate and sinus cycle length

After the establishment of SND, HR decreased and SCL was prolonged compared to baseline (179 ± 13 vs 130 ± 5 bpm, 337.2 ± 25.4 vs 462.7 ± 16.5 ms, respectively) (P < 0.01). In all of the 8 dogs the SCL prolonged more than 100 ms compared to the SCL before SND whereas the average prolongation was 125.4 ± 9.8 ms. The changes in HR and SCL after the 2-hour stimulation were even more pronounced.
(179 ± 13 vs 122 ± 4 bpm and 129.6 ± 4.5 vs 493.8 ± 17.4 ms respectively) (P < 0.01).

4.2. Changes of atrial ERP and its dispersion

As illustrated in Tables 1 and 2, after SND as well as after the 2-hour stimulation, the atrial ERPs were all shortened (P < 0.05) while their dispersion at different PCLs was increased (P < 0.05). Further analysis showed a significant difference in the dispersion of atrial ERPs between PCL250 and PCL350 after the 2-hour stimulation period (30.76 ± 3.06 vs 25.12 ± 3.62 ms, P = 0.014). On the other hand, there were no significant differences in the dispersion of atrial ERPs between PCL350 and PCL250 (29.25 ± 4.43 vs 30.76 ± 3.06 ms, P = 0.861), and between PCL750 and PCL200 (29.25 ± 4.43 vs 25.12 ± 3.62 ms, P = 0.076).

4.3. Induction and duration of atrial fibrillation

Given that 7 atrial sites were used to induce AF in each dog, there were overall 56 sites in the 8 dogs. Before SND, AF was induced by S1–S2 stimulation at 4 sites in 3 dogs. After SND, AF was induced at 12 sites in 6 dogs whereas following the 2-hour stimulation, AF could be induced at 25 sites in 8 dogs. The average duration of inducible AF was longer before as well as after the 2-hour stimulation compared to the duration before SND (33.6 ± 16.1 vs 23.2 ± 12 s, 16.5 ± 4.7 vs 23.2 ± 12 s). The longest duration of AF was also significantly prolonged (117.3 ± 44.2 vs 3.1 ± 2.2 s, 45.7 ± 19.8 vs 3.1 ± 2.2 s) (Table 3). After the 2-hour stimulation, the atrial ERP at AF induction site was shorter compared with the non-inducible site at all PCLs (P < 0.05).

Following the 2-hour stimulation, AF could be induced at 25 sites in 8 dogs (25/56). The 3 animals that were on sustained AF after the 2-hour rapid atrial pacing had more induced stimulation sites than the other 5 animals (12/21 vs 13/35, P < 0.05). The average duration of inducible AF and the longest duration of AF in the 3 dogs on sustained AF after the 2-hour stimulation were also prolonged significantly compared to the others (44.8 ± 13.5 vs 23.2 ± 10.7 s, 148.8 ± 35.2 vs 88.2 ± 29.4 s, P < 0.05).

4.4. Recovery of atrial effective refractory period after the 2-hour stimulation

After the 2-hour stimulation at PCL350, the HRA ERP was recovered to the baseline on the 100th min and thereafter remained stable, but the HLA ERP did not restitute to the baseline until the 140th min. Similarly, after the 2-hour stimulation at PCL250, ERP of HRA was fully recovered to baseline on the 100th min and then remained stable. On the other hand, the HLA ERP began to restitute on the 100th min and fully recovered within 140 min. However, after the 2-hour stimulation at PCL200, the ERP of HRA was changed to the control value on the 60th min and remained stable, while the ERP of HLA began to restitute on the 90th min and did not recover completely until the 100th min (Table 4).

5. Discussion

In this study, using a canine model of SND, we demonstrated that the decreased automaticity and conductivity of sinus node, was associated with shortening of atrial refractoriness. We provided evidence that atrial electrical remodeling is taking place after induction of SND characterized by the shortening of atrial ERP and increasing atrial ERP dispersion. The shortened ERP with slower rates is consistent with the well described loss of physiological adaptation at low rates in experimental models of atrial electrical remodeling [1–3]. Although we did not examine cellular electrophysiologic changes, it could be postulated that ionic channel remodeling was the underlying mechanism.

Furthermore, the inducibility of AF increased after SND and further enhanced after rapid atrial stimulation while the duration of induced AF was significantly prolonged. The recovery of atrial refractoriness after cessation of atrial tachycardia in the LA and RA at different PCLs was not homogeneous. The recovery course of atrial ERP in the LA was significantly delayed compared with that in the RA. It could therefore be suggested that SND induces atrial electrical remodeling which is further aggravated by atrial tachycardia creating an electrophysiologic substrate for AF development and perpetuation.

Brady–tachy syndrome is a common clinical form of SSS, and in general SSS patients are prone to AF. However, the atrial

### Table 1

<table>
<thead>
<tr>
<th>Positions</th>
<th>PCL350</th>
<th>PCL250</th>
<th>PCL200</th>
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<tr>
<td></td>
<td>Before SND</td>
<td>After SND</td>
<td>After stimulation</td>
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<td>HRA</td>
<td>128.3 ± 2.5</td>
<td>120.4 ± 3.1</td>
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<td>MRA</td>
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<td>100.7 ± 2.6</td>
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<td>114.8 ± 2.6</td>
<td>102.8 ± 3.6</td>
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<td>BB</td>
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<td>120.5 ± 2.9</td>
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<td>121.0 ± 1.4</td>
<td>112.5 ± 2.3</td>
<td>96.5 ± 3.5</td>
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<tr>
<td>LLA</td>
<td>120.4 ± 2.2</td>
<td>112.4 ± 2.5</td>
<td>98.3 ± 2.7</td>
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</tbody>
</table>

**Positions:** PCL: pacing cycle length. PCL200, PCL250 and PCL350: pacing cycle lengths of 200 ms, 250 ms and 350 ms.

* Comparison with before sinus node dysfunction, P < 0.05.

### Table 2

<table>
<thead>
<tr>
<th>PCL</th>
<th>Before SND</th>
<th>After SND</th>
<th>After stimulation</th>
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<td>PCL100</td>
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<td>19.38 ± 2.93</td>
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<td>PCL250</td>
<td>13.48 ± 1.81</td>
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<td>30.76 ± 3.06</td>
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<tr>
<td>PCL500</td>
<td>14.50 ± 1.77</td>
<td>17.14 ± 2.14</td>
<td>25.12 ± 3.62</td>
</tr>
</tbody>
</table>

**PCL:** pacing cycle length. PCL200, PCL250 and PCL350: pacing cycle lengths of 200 ms, 250 ms and 350 ms.

* Other notes are as same as Table 1.

### Table 3

<table>
<thead>
<tr>
<th>Sinus function and rapid stimulation</th>
<th>Dogs induced AF</th>
<th>Sites where AF was induced</th>
<th>Average duration of AF (Second)</th>
<th>Longest duration of AF (Second)</th>
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</thead>
<tbody>
<tr>
<td>Before SND</td>
<td>3/8</td>
<td>4/56</td>
<td>2.3 ± 1.2</td>
<td>3.1 ± 2.2</td>
</tr>
<tr>
<td>After SND</td>
<td>6/8</td>
<td>12/56</td>
<td>16.5 ± 4.7</td>
<td>45.2 ± 19.8</td>
</tr>
</tbody>
</table>

**AF:** atrial fibrillation. Notes are as same as Table 1.

* Comparison with Before SND, P < 0.05.

* Comparison with Before SND, P < 0.01.

* Comparison with after SND, P < 0.05.
electrophysiological changes that presumably accompany the SND have not been well studied. Although several experimental and clinical studies have focused on the electrophysiological changes of atrial myocardium in the setting of atrial tachycardia or AF, corresponding data in the setting of SND are limited and inconsistent. Moreover, it is not clear whether atrial tachycardia-induced electrical remodeling is caused by the SND and/or vice versa. For instance, recent evidence suggests that curative ablation of AF results in improved sinus node function pointing to a reverse remodeling process in the sinus node [8]. Sanders et al. [7] showed that in patients with SND but no history of tachyarrhythmias the right atrial ERP is prolonged while the normal physiological rate adaptation of ERP is attenuated or reversed. Interestingly, Luck and Engel showed that there is a prolongation as well as increased dispersion of the right atrial ERP in patients with SND compared to control subjects [9]. However, no difference was observed between subgroups of SND patients with or without AF [9]. It was concluded that the development of AF in SND could be attributed to bradycardia-related inhomogeneous refractoriness which enhances the AF vulnerability of the atrium to atrial premature beats [9]. On the contrary, Dr Sisti et al. [10] retrospectively reviewed patients with SND and found no difference in right atrial ERP, although measurements were performed only at a single site and the control group was not age-matched. Despite this finding, the authors attributed the tendency for AF development to the slowing of local conduction [10].

Atrial tachycardia is known to provoke atrial electrical remodeling, which includes shortening and maladaptation of atrial ERP [5]. In 1995, Morillo et al. [4] reported that after 6 weeks of continuous rapid atrial pacing (400 bpm) in dogs, the right atrial ERP was significantly shortened while this shortening was highly predictive for the induction of sustained AF. In experimental models, atrial tachycardia induces a shortening of the action potential duration (APD) and atrial ERP within a few minutes due to functional alterations in ionic channel activity [11]. Daoud et al. [12] were the first to investigate the atrial electrical remodeling in humans. They studied the induction of AF in 20 patients without structural heart diseases by rapid atrial pacing, and observed AF episodes of average duration of 7 min along with shortened atrial ERP.

We demonstrated that atrial ERPs were shortened at all atrial sites and the dispersion increased significantly in SND dogs. Furthermore, more pronounced changes and increased dispersion of atrial ERP were observed after the 2-hour stimulation period. This inhomogeneity of electrophysiological parameters has also been noted in experimental settings without the presence of SND. Moreover, we observed that the dispersion of atrial ERP was not affected by the PCL at baseline but after the 2-hour stimulation the dispersion of atrial ERP at PCL<sub>250</sub> was greater than that at PCL<sub>200</sub>. Using a dog model, Lee et al. [13] reported that the AF inducibility rate of 9% before rapid stimulation reaches the level of 53% after a 24-hour stimulation period. In our study, the AF inducibility rate was 7.1% before SND, 21.4% after SND, and 44.6% after 2-hour stimulation, indicating that SND contributed significantly to the increased inducibility of AF. After the 2-hour stimulation, atrial ERP in the AF induction site was shorter compared with the non-inducible site. In keeping with previous reports [13,14], our findings indicate that shortening of atrial ERP is related to the inducibility rate of AF.

Current evidence suggests that reverse atrial electrical remodeling is taking place for a few days after the restoration of normal sinus rhythm [1–3]. In experimental models of atrial tachycardia the recovery of atrial ERP is associated with the duration of AF and it can be fully restored to the baseline levels. In a goat model where AF was maintained for 2 to 4 weeks, all electrophysiological changes were fully normalized after 1 week of sinus rhythm [5]. In dogs, the shortened atrial ERP produced by 4 weeks of rapid atrial pacing completely recovered to the baseline value in 2 weeks after termination of pacing [15]. Yu et al. [16] compared the restitution of atrial ERP at different sites and showed that the recovery of atrial ERP in LA was delayed compared to RA and to an area close to Bachman’s bundle facilitating recurrence of AF. Our experimental results showed that the recovery of atrial ERP in LA and RA was non-synchronous. It could therefore be speculated that non-synchronous reversal of electrical remodeling between RA and LA causes exaggerated dispersion of atrial refractoriness particularly in the setting of SND.

6. Limitations

The use of healthy experimental animals in this study precludes the existence of structural heart abnormalities associated with increased atrial stretch and fibrosis. Therefore, we did not measure conduction velocities since this parameter is related to structural remodeling occurring after prolonged periods of atrial tachyarrhythmias and associated with aging, degenerative diseases, heart failure, hypertension, etc. Given that human subjects with SND are often elderly with several concomitant diseases the results of this study cannot be extrapolated to the clinical setting. However, we feel that our model clarifies for the first time the causal relationship between SND and atrial electrical remodeling since previous data were based on human subjects who had comorbidities and therefore only an association and not a causal relationship could be demonstrated. However, we should also acknowledge that our study does not provide a mechanistic explanation since we did not examine ionic remodeling. Finally, programmed electrical stimulation in the 4 dogs in which SND induction was unsuccessful was not performed. Thus, the possibility that the observed electrophysiological changes were related to the procedure and not solely to the SND cannot be totally excluded.

7. Conclusion

Experimental SND induces atrial electrical remodeling which is further aggravated by atrial tachycardia. Of note, AF inducibility and duration in this model is significantly increased, suggesting that SND and atrial tachycardia create a substrate for AF initiation and perpetuation. Taking into account that the elderly population with SND is continuously expanding, our experimental model could be a useful tool for the examination of innovative therapeutic strategies targeting at the attenuation of electrical remodeling in order to reduce the AF burden.
Acknowledgement

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [17].

References