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Original Article

Reversibility of both sinus node dysfunction and reduced HCN4 mRNA expression level in an atrial tachycardia pacing model of tachycardia-bradycardia syndrome in rabbit hearts

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Abstract: Objective: The aim of this study was to investigate potential reversible changes in sinus node function and mRNA expression levels of hyperpolarization-activated, cyclic nucleotide-gated ion channel subunit 4 (HCN4) in a tachycardia pacing model in rabbits. Methods: A total of 45 adult New Zealand white rabbits were randomized into the following three groups (n=15): pacing only, pacing-recovery and control. Following open thoracotomy, temporary pacing leads were attached to the right atrium. In the pacing only group, rapid atrial pacing was initiated at a rate of 350 stimuli per minute for 8 hours a day, continuing for 7 days. In the pacing-recovery group, the same pacing protocol was delivered, but pacing was then stopped for 7 days. In the control group, no tachycardia pacing was delivered. The following parameters were measured before and after intervention, and compared between the groups: resting heart rate, intrinsic heart rate (measured after metoprolol and atropine administration) and corrected sinus node recovery time (CSNRT). The rabbits were then killed, following which the sinus nodes were excised and used for testing of hyperpolarization-induced cyclic nucleotide gated (HCN) 4 mRNA expression. Results: In the pacing only group, the resting heart rate and intrinsic heart rate were decreased (219.71 ± 3.59 vs. 275.86 ± 13.31 bpm and 202.00 ± 4.76 vs. 227.14 ± 4.98 bpm, respectively; $P < 0.05$) and CSNRT was prolonged (96.00 ± 3.56 vs. 72.00 ± 2.31 ms) after tachycardia pacing. In the intervention-recovery group, similar changes in resting heart rate, intrinsic heart rate and CSNRT were observed after tachycardia pacing. After 7 days of recovery, all three parameters returned to normal values (resting heart rate: 264.67 ± 9.82 vs. 222.56 ± 5.90 bpm; intrinsic heart rate 219.33 ± 5.67 vs 213.86 ± 3.29 bpm; CSNRT 76.33 ± 5.89 vs. 99.44 ± 6.17 ms, all $P < 0.05$). The HCN4 mRNA expression level in the pacing only group was reduced compared to the control group (0.37 ± 0.04 vs 0.65 ± 0.04, $P < 0.05$). This recovered from 0.35 ± 0.04 to 0.60 ± 0.04 in the pacing-recovery group. Conclusion: Rapid atrial pacing led to reversible sinus node dysfunction, which was partly due to return of HCN4 expression. Electrophysiological remodeling of I_f may be important in sinus node dysfunction observed in tachycardia-bradycardia syndrome.

Keywords: Cardiac electrophysiological, rapid atrial pacing, sinus node dysfunction, hyperpolarization activated cyclic nucleotide gated cation channel 4

Introduction

Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice. Over the past decade, radiofrequency catheter ablation (RFCA) has emerged as a promising treatment option, with a significant number of patients reverting back to sinus rhythm thereafter. However, in some cases, sinoatrial node (SAN) dysfunction was observed following AF termination, which could result in tachycardia-bradycardia syndrome (TBS). These patients may require pacemaker implantation, placing a heavy financial burden on the healthcare system.

The mechanisms of TBS have not been fully elucidated, but several studies have implicated atrial arrhythmias as the underlying cause of SAN dysfunction [1, 2]. For example, structural

Table 1. The primer design of HCN4 and GADPH

Gene	Primer Sequences	Annealing temperature (°C)	Product size (bp)
HCN4	Forward 5'-AGGAGATCATCAACTTCAACTG-3'	56	165
	Reverse 5'-AGTACATCTTCTTGCCGATGGT-3'		
GADPH	Forward 5'-GCTTTTAACTCTGGCAAAGTG-3'	56	390
	Reverse 5'-GATGATGACCCTTTTGGCTC-3'		

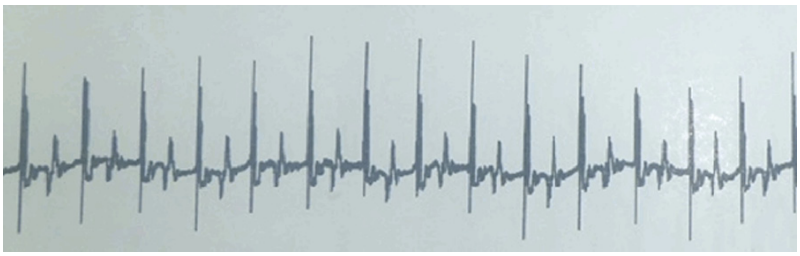

Figure 1. The electrocardiogram of rapid atrial pacing (350 bpm).

abnormalities of SAN have been observed in patients with AF [3]. There is also increasing evidence of a reversible component due to electrophysiological remodeling. Automaticity of the SAN is dependent on both voltage- and calcium-dependent clock mechanisms [4]. The former is mediated by the funny current (I_f), which flows through the channel encoded by the hyperpolarization-activated, cyclic nucleotide-gated ion channel gene (HCN) [5]. Yeh YH et al. used atrial tachycardia pacing to induce sinoatrial node (SAN) dysfunction for modelling TBS [6]. These authors noted If downregulation and decreased mRNA transcript levels of HCN subtypes 2 and 4. However, reversibility of these changes was not examined. In this study, therefore, we examined the nodal function as reflected by resting and intrinsic heart rates, HCN4 mRNA expression levels in a rabbit model using tachycardia pacing. We demonstrate that recovery of resting and intrinsic heart rates and HCN4 mRNA expression levels after cessation of tachycardia pacing.

Materials and methods

Experimental animals

This study complied with the National Institutes of Health guidelines and was approved by the Animal Research Ethics Committee of the Shanghai Tongji Hospital. A total of 45 New Zealand White rabbits aged 12 weeks and weighed between 2.5 and 3 kg were used in this study. They were randomly divided into three groups: pacing only, pacing-recovery and control (n=15 each).

Implantation of a temporary pacemaker electrode

In the both pacing only and pacing-recovery groups, the rabbit was anesthetized by administering sodium pentobarbital intravenously (30 mg/kg). A median sternotomy approach was used to expose the heart, which allowed introduction of a temporary pacing electrode (YS99-01). The negative pole was fixed to the free wall of the right atrium, whereas the positive pole was placed outside. The latter was connected to electrophysiological heart stimulator to deliver tachycardia pacing at 350 beats per minute (bpm) for 8 hours each day for 7 days. The pacing-recovery group was allowed a 7-day recovery period.

Sinoatrial node (SAN) function

Three indices were used to represent SAN function: (1) resting heart rate, which was measured for 10 seconds during an inactivate state of the animals; (2) intrinsic heart rate, measured 10 minutes after intravenous injection of the beta blocker metoprolol (0.2 mg/kg) and muscarinic receptor antagonist atropine (0.04 mg/kg); (3) corrected sinus node recovery time (CSNRT), measured using a stepwise increase S1-S1 protocol. CSNRT was defined as the difference between the sinus node recovery time (SNRT) and the R-R interval. These parameters were assessed before and the 1, 3, 7 day after pacing for the intervention only and intervention-recovery groups, and at the same time points for the control group.

Excision of SAN tissue

Rabbits were killed to allow excision of SAN from the heart using the following method. Cuts were made along atrioventricular groove

Table 2. Resting heart rate, intrinsic heart rate and CSNRT in the pacing only group (n=11)

Index	Before pacing	Days after pacing		
		1	3	7
Resting heart rate (bpm)	275.86±13.31	244.57±5.44*	230.86±6.64*	219.71±3.59*
Intrinsic heart rate (bpm)	227.14±4.98	221.00±3.96*	213.86±3.29*	202.00±4.76*
CSNRT (ms)	72.00±2.31	80.42±4.54*	87.00±4.80*	96.00±3.56*

*Indicates statistically significant at $P < 0.05$.

Table 3. Resting heart rate, intrinsic heart rate and CSNRT in the pacing-recovery group (n=13)

Index	Before pacing	Pacing for 7 days	Stopping pacing for 7 days
Resting heart rate (bpm)	278.11±7.10	222.56±5.90*	264.67±9.82*,#
Intrinsic heart rate (bpm)	224.67±5.63	201.44±4.03*	219.33±5.67*,#
CSNRT (ms)	72.11±5.01	99.44±6.17*	76.33±5.89*,#

*Denotes comparison between the pacing-recovery and pacing only groups; #denotes comparison before and after pacing in the pacing-recovery group.

between the left and right ventricles. This was followed by separation of the right atrium from the tricuspid valve annulus. The tissue under the superior vena cava near the crista terminals was considered SAN tissue (4 × 5 mm in size). This was immediately placed in Ca-free Tyrode solution (contained NaCl 137 mmol/L, KCl 5.0 mmol/L, NaH2PO4 1.4 mmol/L, NaHCO3 12 mmol/L, MgSO4 1 mmol/L, Glucose 10 mmol/L, HEPES 5 mmol/L, pH 7.30), and then in a liquid nitrogen freezer at -70°C.

Measurement of HCN4 mRNA levels by RT-PCR

The mRNA expression levels for HCN4 were determined according to operator instructions using RT-PCR. The Kodak gel electrophoresis analytic system was used to scan the electrophoresis bands. The optical density (OD) of HCN4 and phosphoglyceraldehyde dehydrogenase (GADPH, reference substance) was measured, which allowed calculation of their OD ratio. The primer sequences, reaction conditions and product size are listed in **Table 1**.

Statistical analysis

Statistical analysis was performed using SPSS for Windows (Version 17.0). Continuous data were expressed as mean±standard deviation. Comparisons were made using Student's t-test for paired and unpaired data, as appropriate. For all tests, $P < 0.05$ was considered statistically significant.

Results

Atrial tachycardia pacing model

Pacemaker insertion was performed in all 45 rabbits accepted operation. Of these, 8 rabbits died during the procedure (3 each in intervention only and control groups, 2 in intervention-recovery group) due to left atrial rupture, and therefore could not be used for subsequent experimentation. The success rate of operation was 82.2%. 1 rabbits in the pacing only group chewed off the wire a day after the operation. **Figure 1** showed a typical trace of the electrocardiogram when atrial tachycardia pacing was delivered at a rate of 350 bpm.

Sinus node dysfunction after atrial tachycardia pacing

Three indices were used to represent sinus node function: resting heart rate, intrinsic heart rate and corrected sinus node recovery time (CSNRT). In the pacing only group, both resting heart rate and intrinsic heart rate progressively decreased, whereas CSNRT progressively increased, at days 1, 3 and 7 after initiation of pacing ($P < 0.05$). This is summarized in **Table 2**.

In the pacing-recovery group, progressive decreases in the resting heart rate and intrinsic heart rate, and increases in the CSNRT, were observed, as in the pacing only group. After cessation of pacing for 7 days, all three parameters recovered incompletely. This is summarized in **Table 3**.

HCN4 expression

The relative expression levels of HCN4 mRNA were highest in the control group, taking a value

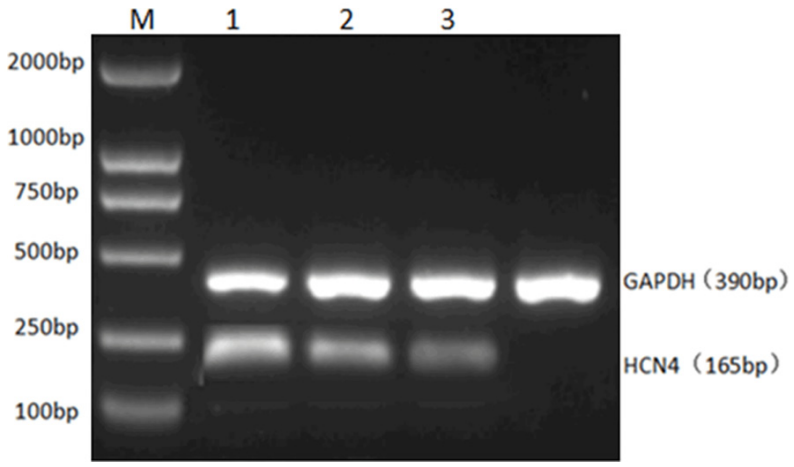

Figure 2. The electrophoresis bandings for HCN4 mRNA expression (1 = control group, 2 = pacing-recovery group, 3 = pacing only group).

of 0.65 ± 0.04. In the pacing only group, this was reduced to 0.37 ± 0.04, representing a 42% decrease, when compared to control. In the pacing-recovery group, HCN4 mRNA expression level was higher than the pacing only group, taking a value of 0.60 ± 0.04. This represented an incomplete recovery, as this was still significantly smaller than the control group ($P < 0.05$). The electrophoresis bandings for HCN4 mRNA expression in these three groups are shown in **Figure 2**.

Discussion

SAN automaticity is dependent upon both voltage- and calcium-dependent mechanisms [7]. The former is represented by the funny current (I_f) flowing through hyperpolarization-activated, cyclic nucleotide-gated (HCN) ion channels [5]. These channels have several unusual characteristics, such as activation on hyperpolarization, permeability to both sodium and potassium ions, modulation by intracellular cyclic AMP, and a small single channel conductance [8]. Four HCN isoforms have been identified thus far, with immunohistochemical experiments showing subtype 4 as the predominant isoform in SAN tissue [9]. HCN4 mutation is known to cause sick sinus syndrome [10]. HCN4 gene knockout mice showed severe sinus bradycardia complicated by atrioventricular block [11].

Sinoatrial node (SAN) dysfunction and atrial tachycardia are often observed together [12]. This may manifest clinically as tachycardia-bradycardia syndrome (TBS) [2]. Clinical studies have suggested atrial fibrillation, as a cause of SAN dysfunction through overdrive suppression, as reflected in prolongations of corrected sinus node recovery time (CSNRT) and sinoatrial conduction time (SACT) [13, 14]. In other words, the tachycardia is responsible for the bradycardic manifestations. Animal studies have been performed to investigate TBS further [15]. The molecular mechanisms were shown to involve decreased amplitudes of the funny current (I_f) using patch clamping, consistent with the decreased mRNA transcript levels for HCN2 and HCN4 observed by RT-PCR [6]. Electrical remodeling is thought to be the main mechanism underlying tachycardia-induced SAN dysfunction [16-18]. Previous studies have found recovery of SAN function after the ablation for atrial fibrillation [19-22]. In AF patients with sinus pause, ablation procedures led to recovery of minimum, maximum and average heart rates to levels comparable to before treatment, with either abolition of sinus pause or decreased duration of the pause [20, 21]. Although recovery of SAN function after resolution of atrial tachycardia is well-known [15, 20, 23], the molecular changes accompanying such reversibility is less well-studied.

In this study therefore, we used tachycardia pacing to induce SAN dysfunction using a rabbit model, thereby examining its reversibility. This was correlated with alterations in HCN4 mRNA transcript levels. A temporary pacemaker was implanted using a thoracoscopic approach with a high success rate (82.2%), suggesting that this technique is a feasible for wider experimental studies. Tachycardia pacing was delivered for 7 days followed by a 7-day of pacing free period. At the end of this period, both resting and intrinsic heart rates recovered, which was accompanied by reversible increase of mRNA expression levels for HCN4 for the first time in rabbit hearts.

Limitations and future avenues

There are several limitations of this study. Firstly, the voltage clock mediated by the I_f is only one mechanism underlying automaticity of

the SAN. There are additional contributions from other currents [24] and the calcium clock mechanism. The latter is mediated by spontaneous release of calcium content from the sarcoplasmic reticulum into the cytosol via the ryanodine receptor [4]. This in turn results in activation of the sodium-calcium exchanger [25]. In tachycardia pacing-induced AF of canine hearts, ryanodine receptor type 2 (RyR2) was downregulated [26]. A future study can examine whether these channels are similarly reduced in the rabbit model, and if so, also its potential reversibility. Secondly, patch clamp study is needed to confirm changes in I_f amplitude and histology is needed to explore the roles of fibrosis. Finally, our study found that SAN function and HCN4 expression did not recover fully. It may well be that HCN4 mRNA transcript levels could increase beyond 7 days of recovery period.

Conclusion

Atrial tachycardia pacing induces SAN dysfunction and decreased expression levels of HCN4 mRNA transcript. Both are reversible upon cessation of pacing. Remodeling of funny current may play an important role in TBS.

Disclosure of conflict of interest

None.

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