Ionic mechanisms underlying region-specific remodeling of rabbit atrial action potentials caused by intermittent burst stimulation

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BACKGROUND Pulmonary veins (PVs) and the coronary sinus (CS) play pivotal roles in triggering some episodes of atrial fibrillation. In isolated rabbit right or left atrial preparations, a 3-hour intermittent burst pacing protocol shortens action potential duration (APD) in CS and PV, but not in sinus node (SN) and left Bachmann bundle (BB) regions.

OBJECTIVE The purpose of this study was to use patch clamp techniques to study the rapidly inactivating (I_{to}) and sustained (I_{sus}) K^+ currents as well as Ca^{2+} currents (I_{Ca}) in cells dispersed from intermittent burst pacing and sham PV, BB, CS, and SN regions to determine whether changes in these currents contributed to APD shortening.

METHODS Real-time polymerase chain reaction was performed for transient outward K^+ and Ca^{2+} channel subunit mRNAs to determine if intermittent burst pacing affected expression levels.

RESULTS I_{to} densities were unaffected by intermittent burst pacing in PV and Bachmann bundle cells. mRNA levels of $K_V4.3$, $K_V4.2$, $K_V1.4$, and KChIP2 subunits of I_{to} in both regions were stable. In CS cells, I_{to} densities in intermittent burst pacing were greater than in sham ($P < .05$), but there were no parallel mRNA changes. I_{Ca} density of PV cells was reduced from 14.27 ± 2.08 pA/pF (at −5 mV) in sham to 7.52 ± 1.65 pA/pF in intermittent burst pacing PV cells ($P < .05$) due to a significant shift in voltage dependence of activation. These results were seen in the absence of mRNA changes in α_{1C} and α_{1D} Ca^{2+} channel subunits. In contrast, intermittent burst pacing had no effect on Ca^{2+} current densities and kinetics of CS cells, but decreased α_1C and α_1D mRNA levels.

CONCLUSION There is region-specific remodeling of I_{to} and I_{Ca} by intermittent burst pacing protocols in rabbit atrium. Increased I_{to} in CS cells could account for the APD shortening observed with intermittent burst pacing, whereas an intermittent burst pacing-induced shift in voltage dependence of activation may contribute to APD shortening in PV cells.

KEYWORDS Atrial fibrillation; Remodeling; Ionic currents; Pacing

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Introduction

Pulmonary veins (PV)[1,2] and the coronary sinus (CS)[3,4] are origins of ectopic foci in the initiation and perpetuation of some episodes of clinical atrial fibrillation (AF). Attuel et al[5] showed that bigeminal pacing induced bursts of AF in patients with a history of paroxysmal AF but not in controls. The reasons for this finding remain unknown. In an experimental model using isolated right and left normal rabbit atria, an intermittent burst pacing protocol applied in PV or CS regions induced significant shortening of action potential duration (APD) at these sites.[6] The result was sustained changes in atrial repolarization gradients that created an arrhythmogenic substrate. This same intermittent burst pacing protocol applied to the sinus node (SN) and left BB regions had no sustained effect on repolarization. Importantly, the intermittent burst pacing protocol used in these experimental studies was developed to mimic the impulse initiation patterns of intermittently firing foci in CS and PV.

Intermittent burst pacing-induced APD shortening could be due to either a reduction in Ca^{2+} currents and/or an upregulation of K^+ currents. Although many have reported a marked decrease in L-type Ca^{2+} ($I_{Ca,L}$) and transient outward K^+ currents (I_{to}) in atrial cells from long-lasting AF or sustained rapid atrial pacing[7–9] and those from humans in chronic AF,[10,11] no data are available regarding the ionic mechanisms underlying intermittent burst pacing-induced APD shortening in cells of the PV and CS regions.

The purpose of this study was to determine whether $I_{Ca,L}$ and I_{to} are altered in function/density in atrial myocytes dispersed from PV and CS regions in intermittent burst pacing left and right atrial preparations and in intermittent burst pacing SN and left Bachmann bundle atrial cells. Regional alterations in ion channel expression also were studied.

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Materials and methods

This investigation conforms with the *Guide for the Care and Use of Laboratory Animal* published by the National Institutes of Health (NIH Publication No. 85-23, 1996).

Atrial preparations

Atrial preparations used in these studies are identical or equivalent to those used to perform the tissue action potential studies of Sosunov et al.[6] Young mature male New Zealand White rabbits (N = 23, age 3 months, weight 2–2.5 kg) were anesthetized with sodium pentobarbital (30 mg/kg IV) and heparinized (1,000 U/kg). The hearts were excised and placed in Tyrode's solution equilibrated at 37°C with 95% O_2 and 5% CO_2. The Tyrode's solution contained the following (in mM): NaCl 131, $NaHCO_3$ 18, KCl 4, $CaCl_2$ 0.9, $MgCl_2$ 0.5, NaH_2PO_4 1.8, and dextrose 5.5. Separate preparations of right and left atria were isolated, opened to expose the endocardial surface, pinned to the bottom of a 4-mL tissue bath, and superfused with Tyrode's solution at a rate of 12 mL/min. The bath was connected to ground via a 3 M KCl/Ag/AgCl junction. Two bipolar Teflon-coated silver stimulating electrodes were attached to each preparation: near the sinus node (SN) and in the CS ostium in the right atrium and near left Bachmann bundle and in the ostia of right or left inferior PVs in the left atrium. (see Figure 1 in Sosunov et al[6]).

Intermittent burst pacing protocol

Stimulation protocols were described previously.[6] For stimulation, standard techniques were used to deliver square-wave pulses 1.0 ms in duration and 1.5 times threshold through bipolar polytetrafluoroethylene (PTFE)-coated silver electrodes. Burst pacing protocol for intermittent burst pacing preparations was started after 1 hour of superfusion in control Tyrode's solution while pacing from the SN region (right side) or Bachmann bundle region (left side). Preparations were driven for 4.5 mins of each 5-minute period from SN or Bachmann bundle regions at cycle length (CL) = 400 ms (S1, normal spread of excitation) followed by 30 seconds of stimulation from CS or PV at CL = 200 ms (S2, ectopic focus). This pattern of pacing was applied for 3 hours. For sham preparations, stimulation at CL = 400 ms was used to drive preparations from SN and BB for the entire 3 hours. At completion, tissues from intermittent burst pacing and sham preparations were harvested for either polymerase chain reaction analyses or myocyte voltage clamp experiments. Consistent with our previous findings,[6] 3-hour intermittent burst pacing but not sham pacing accelerated action potential repolarization in CS and PV atrial preparations (Table 1).

Real-time polymerase chain reaction

PV, Bachmann bundle, CS, and SN regions[6] were carefully dissected from all tissue preparations and quickly frozen in liquid nitrogen. Total RNA from these regions was individually extracted using the RNeasy midi-kit (Qiagen, Valencia, GA). First-strand cDNA was generated from 0.2 μg of total RNA using SuperScript First-Standard Synthesis System (Invitrogen, Carlsbad, CA). Real-time polymerase chain reaction was performed using a LightCycler (Roche, Mannheim, Germany) with a reaction mixture composed of 2 μL of the cDNA mixture, 2 μL of LightCycler–Faststart DNA master SYBR Green I (Roche), 4 mmol/L Mg^{2+}, and 0.5 μmol/L of each primer in a final volume of 20 μL

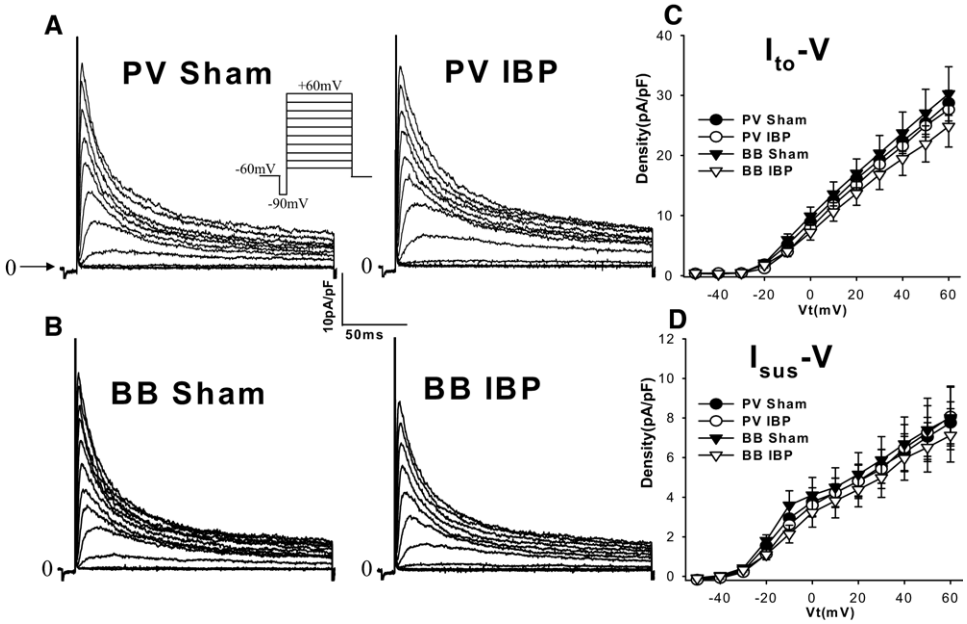

Figure 1 Current–voltage (I–V) relation curves of I_{to} and I_{sus} in pulmonary vein (PV) and Bachmann bundle (BB) cells. **A, B:** Representative current recordings in cells isolated from PV **(A)** and BB **(B)** regions. Data were obtained with a prepulse potential of −90 mV from a holding potential of −60 mV with 10-mV depolarizing steps to voltages from −50 to +60 mV. **C, D:** I–V relation for I_{to} **(C)** and I_{sus} **(D)** as a function of test potential. Data are given as mean ± SEM (PV sham, n = 13; PV IBP, n = 14; BB sham, n = 9; BB IBP, n = 7). IBP = intermittent burst pacing.

Table 1 Effects of 3-hour IBP and sham pacing on APD_{90} in isolated preparations of right and left atrium

	Right atrium				Left atrium			
	IBP		Sham		IBP		Sham	
	(N = 15)		(N = 16)		(N = 19)		(N = 27)	
	SN	CS	SN	CS	BB	PV	BB	PV
Control	103 ± 5	87 ± 4	107 ± 6	90 ± 4	90 ± 4	107 ± 6	92 ± 4	109 ± 5
Pacing	105 ± 6	67 ± 5*	106 ± 5	89 ± 4	91 ± 3	87 ± 5*	91 ± 5	108 ± 4

APD_{90} = action potential duration to 90% repolarization (in milliseconds); BB = Bachmann bundle; Control = before application of IBP pacing protocol; CS = coronary sinus; IBP = intermittent burst pacing; N = number of preparations; Pacing = after application of IBP pacing protocol; PV = pulmonary vein; Sham = sham pacing; SN = sinus node.
*P <.05 vs respective control.

(Table S1, Data Supplement). Fluorescence was measured at the end of each extension step, and units were calculated based on a standard curve generated from serially diluted samples. Data were normalized to data for cyclophilin A from same region. Product sequences were verified by sequencing at the DNA facility of Columbia University (New York, NY, USA).

Cell preparation

Atrial preparations were removed and immersed in Tyrode's solution. PV, Bachmann bundle, CS, and SN regions[6] were carefully dissected free with a dissection microscope. A small piece of tissue from each region was placed in a 60-mm-diameter dish containing 5 mL of enzyme solution (Worthington type II collagenase, 4 mg/mL). The enzyme solution was composed of the following (in mM): KCl 5.4, KH_2PO_4 0.4, $MgSO_4$ 16.6, NaCl 137, $NaHCO_3$ 4.3, Na_2HPO_4 0.47, glucose 5.6, phenol red 0.056, and amino acid solution 1% (MEM) (Gibco, Carlsbad, CA). pH was adjusted to 6.72 with NaOH. The dish containing the tissue was placed in a 37°C rotary shaker bath and agitated at a rate 1–2 cycles per second for 35 minutes. The tissue piece then was placed in a tube containing 3–4 mL of HEPES-buffered salt solution with 145 mM potassium glutamate and 5.7 mM $MgCl_2$ (pH 6.72), stored at 37°C for 10 minutes. The softened tissue was dispersed into single cells by a series of 20 gentle triturations, after which the tissue fragments were allowed to settle and the supernatant drawn off and saved. Fresh solution was added, and another trituration cycle was performed. Dispersed cells in saved supernatant were centrifuged and resuspended in an MEM solution that was changed every 15 minutes to one containing increasing concentrations of Ca^{2+} (0–0.5 mM). Cells were stored at room temperature until studied.

Patch clamp recording

For electrophysiologic study, an aliquot of cells was transferred onto a polylysine-coated glass coverslip placed at the bottom of a 0.5-mL tissue chamber that had been mounted on the stage of a Nikon inverted microscope (Nikon Diaphot, Tokyo, Japan). Cells were continuously superfused (2–3 mL/min) with normal Tyrode's solution containing the following (in mM): NaCl 137, $NaHCO_3$ 24, NaH_2PO_4 1.8, $MgCl_2$ 0.5, $CaCl_2$ 2.0, KCl 4.0, dextrose 5.5 (pH = 7.4). The solution was bubbled with 5%CO_2 and 95%O_2. K^+ and Ca^{2+} currents were studied using solutions and clamp protocols as described previously.[12,13] There was no difference in cell capacitance among cells from four regions (PV, BB, CS, and SN). Intermittent burst pacing did not affect the cell capacitance in each region. In PV, cell capacitance was 72.0 ± 5.9 pF in sham (n = 23) and 71.7 ± 5.0 pF in intermittent burst pacing (n = 22, P = NS). In Bachmann bundle, cell capacitance was 69.5 ± 4.6 pF in sham (n = 13) and 73.6 ± 10.8 pF in intermittent burst pacing (n = 9, P = NS). In CS, cell capacitance was 71.5 ± 6.4 pF in sham (n = 12) and 77.0 ± 7.6 pF in intermittent burst pacing (n = 12, P = NS). In SN, cell capacitance was 75.5 ± 6.9 pF in sham (n = 14) and 73.9 ± 5.8 pF in intermittent burst pacing (n = 12, P = NS).

Drugs

C9356, a putative selective blocker of $K_V1.5$,[14] was a gift from Cardiome Corp (Vancouver, BC, Canada). The stock solution of C9356 was made in dimethylsulfoxide.

Statistical analysis

Data are given as mean ± SEM. n is the number of cells from sham PV, intermittent burst pacing PV, sham CS, intermittent burst pacing CS; sham Bachmann bundle, intermittent burst pacing Bachmann bundle; and sham SN and intermittent burst pacing SN regions. Analysis of variance was completed first. Then comparison between group means was performed using Student's t-test, if appropriate. P <.05 was considered significant.

Results

I_{to} and I_{sus}

Representative current recordings of I_{to} and I_{sus} from PV and BB cells from sham and intermittent burst pacing preparations are shown in Figures 1A and 1B. Summary data for current–voltage (I–V) relationships are shown in Figures 1C and 1D. I_{to} and I_{sus} densities were not affected by intermittent burst pacing in PV and BB cells. Data are summarized in Table S2 (Data Supplement). Figures 2A and 2B show

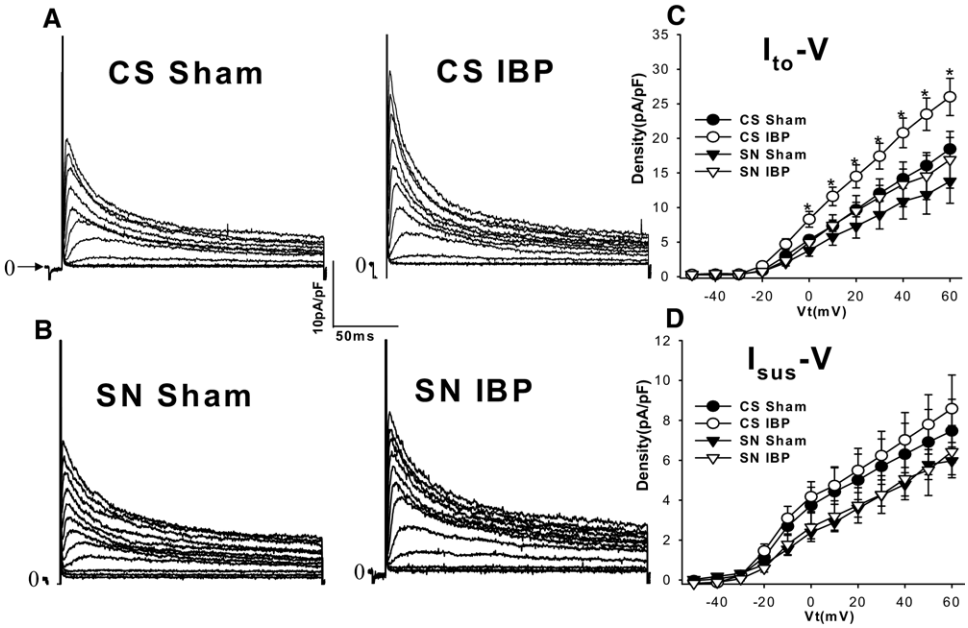

Figure 2 Current–voltage (I–V) relation curves of I_{to} and I_{sus} in coronary sinus (CS) and sinus node (SN) cells. **A, B:** Representative current recordings in cells isolated from CS **(A)** and SN **(B)** regions. Data were obtained with a prepulse potential of −90 mV from a holding potential of −60 mV with 10-mV depolarizing steps to voltages from −50 to +60 mV. **C, D:** I–V relation for I_{to} **(C)** and I_{sus} **(D)** as a function of test potential. Data are given as mean ± SEM (CS sham, n = 8; CS IBP, n = 6; SN sham, n = 7; SN IBP, n = 6). IBP = intermittent burst pacing.

tracings of I_{to} and I_{sus} from CS and SN cells from sham and intermittent burst pacing preparations. Summary data are shown in Figures 2C and 2D. Interestingly, intermittent burst pacing significantly increased I_{to} density in CS cells within and above the physiologically relevant range of potentials. In contrast, there was no effect of intermittent burst pacing on SN cells. Further, I_{sus} density was not affected by intermittent burst pacing in both CS and SN cells (Figure 2D).

Whereas the upregulated I_{to} in CS cells may contribute to the APD shortening observed with intermittent burst pacing, voltage-dependent outward currents appear not to contribute to the intermittent burst pacing-remodeled action potential in the PV region.

Because $K_V1.5$ has been thought to be atrial-specific I_{Kur} in humans, dogs, and rats,[14,15] we evaluated $K_V1.5$ currents using the putative specific $K_V1.5$ blocker C9356.[14] Figures 3A and 3B (top) show the original tracings in the absence and presence of 10 μM C9356 in PV and Bachmann bundle sham and intermittent burst pacing cells. Average data are shown in Figures 3C and 3D (top). There are no significant differences between sham and intermittent burst pacing in both PV and Bachmann bundle cells (Table S2, Data Supplement). Similarly, as in Figures 3A and 3B (bottom), there are no significant differences in C9356-sensitive and C9356-insensitive currents between CS and SN sham and between CS and SN intermittent burst pacing cells (Table S3, Data Supplement). Thus, an atrial-specific current sensitive to C9356 is not regulated by intermittent burst pacing in rabbit atrial cells from the four regions studied.

To evaluate possible mechanisms involved in the intermittent burst pacing-related changes in I_{to}, the voltage-dependent and kinetic properties were determined and compared (Tables S4 and S5, Data Supplement). The intermittent burst pacing protocol had no effect on inactivation parameters in any region but produced a modest slowing in recovery of I_{to} in intermittent burst pacing CS cells (Table S5).

Effect of intermittent burst pacing on I_{to} and I_{sus} subunit mRNA expression

$K_V4.3$, $K_V4.2$, and $K_V1.4$ are thought to contribute to I_{to} in rabbit atrium.[16] However, $K_V1.5$ expression in rabbit atrium has not been reported. Furthermore, one K_V channel accessory subunit, the K_V channel interacting protein (KChIP2),[17] has been shown to regulate K_V4, $K_V1.4$, and $K_V1.5$.[18] After 3-hour intermittent burst pacing, mRNA levels of all these I_{to} subunits in PV and Bachmann bundle regions were stable (Figure S1, Data Supplement), consistent with a lack of effect of intermittent burst pacing on I_{to} and I_{sus} in PV and left Bachmann bundle (Figure 1). However, in CS region, $K_V4.3$, $K_V1.4$, and $K_V1.5$ mRNA expression levels were significantly reduced by 3-hour intermittent burst pacing (Figure 4), whereas $K_V4.2$ and KChIP2 mRNA levels were unchanged (Figure S1, Data Supplement). Clearly, this result does not parallel the upregulated I_{to} in CS cells (Figure 2). In SN region, all subunit mRNA expression levels except $K_V1.5$ were unaffected by intermittent burst pacing (Figures 4 and S1). We identified $K_V1.5$ mRNA expression in rabbit atrium (Figure 4), and $K_V1.5$ mRNA levels displayed significant and general reduction after intermittent burst pacing in all regions but Bachmann bundle. Yet currents sensitive to a putative $K_V1.5$ chan-

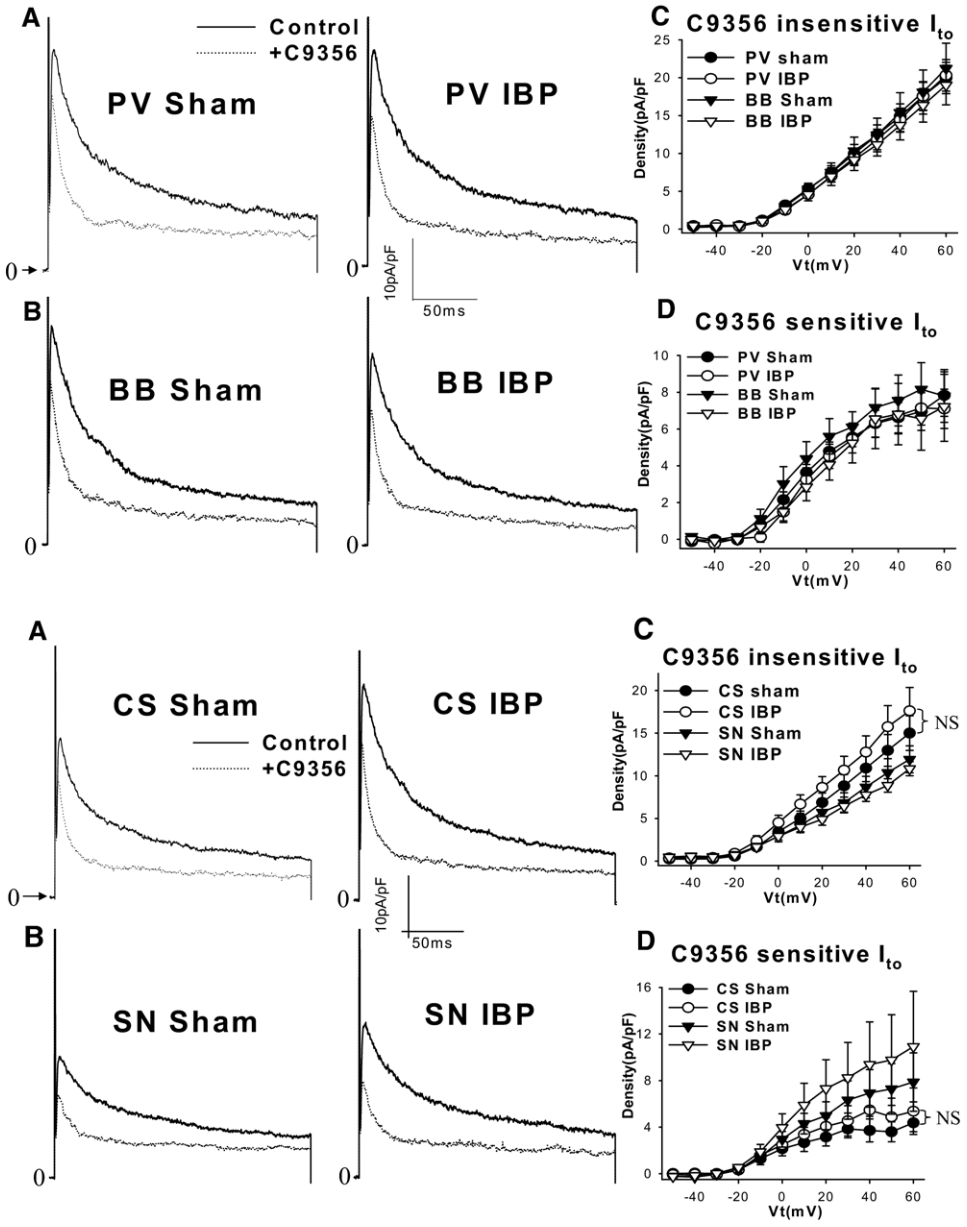

Figure 3 **Top. A, B:** Representative recordings at Vt +40 mV from Vh −60 mV showing the effect of C9356 on I_{to} and I_{sus} in cells isolated from pulmonary vein (PV; **A**) and Bachmann bundle (BB; **B**) regions. **C, D:** Current–voltage (I–V) relations of C9356-insensitive **(C)** and C9356-sensitive **(D)** I_{to} currents. Data are given as mean ± SEM (PV sham, n = 12; PV IBP, n = 8; BB sham, n = 6; BB IBP, n = 6). **Bottom. A, B:** Representative recordings at Vt +40 mV from Vh −60 mV showing the effect of C9356 on I_{to} and I_{sus} in cells isolated from coronary sinus (CS; **A**) and sinus node (SN; **B**) regions. **C, D:** I–V relations of C9356-insensitive **(C)** and C9356-sensitive **(D)** I_{to} currents. Data are given as mean ± SEM (CS sham, n = 5; CS IBP, n = 6; SN sham, n = 6; SN IBP, n = 6). IBP = intermittent burst pacing.

nel blocker showed no change in all regions (Figure 3D, top/bottom).

I_{Ca}

Figures 5A and 5B show representative tracings of I_{Ca} in PV and left Bachmann bundle cells from sham and intermittent burst pacing preparations. Summary data are shown in Figures 5C and 5D. Note that there were no significant changes in peak I_{Ca} density from sham and intermittent burst pacing of PV and Bachmann bundle cells. However, in PV cells, the I–V curve was shifted in a depolarizing direction by intermittent burst pacing. At −5 mV, I_{Ca} density of PV cells was 14.27 ± 2.08 pA/pF in sham (n = 15) and 7.52 ± 1.65 pA/pF in intermittent burst pacing (n = 14, $P < .05$). Activation curve in PV cells was shifted to the depolarized direction by intermittent burst pacing (Figure 5E). $V_{0.5}$ was −7.5 ± 2.2 mV in sham and 2.8 ± 2.1 mV in intermittent burst pacing ($P < .5$; Table S6, Data Supplement). Unlike PV cells, I_{Ca} of Bachmann bundle cells was unaffected by intermittent burst pacing. Furthermore, there were no significant changes in voltage dependence of inactivation and recovery from inactivation in both PV and Bachmann bundle cells by intermittent burst pacing (Table S8, Data Sup-

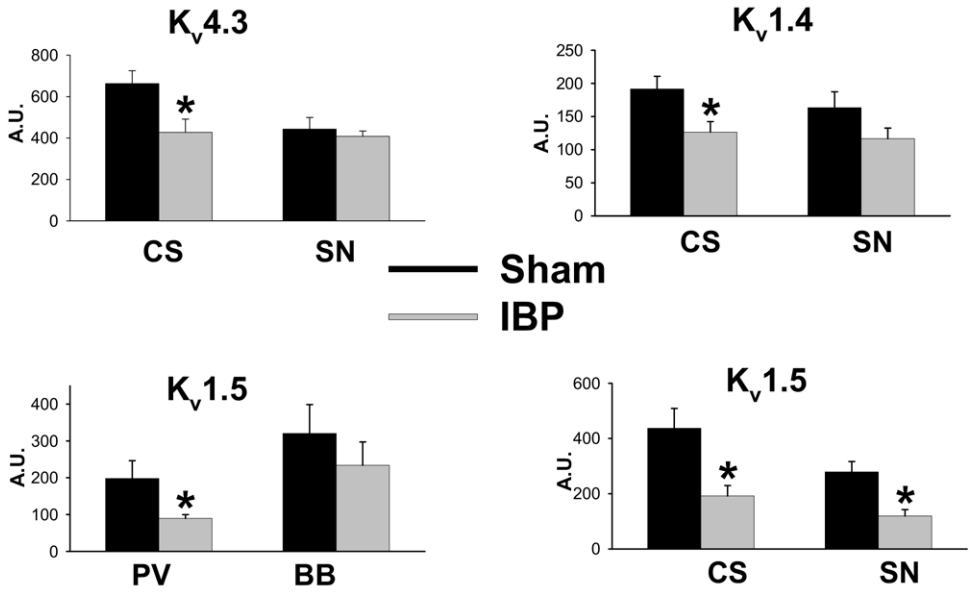

Figure 4 Effects of intermittent burst pacing (IBP) on mRNA levels of $K_V4.3$, $K_V1.4$, and $K_V1.5$ in coronary sinus (CS), sinus node (SN), pulmonary vein (PV), and Bachmann bundle (BB). A.U. is a relative arbitrary unit. Data are given as mean ± SE (n = 7 rabbits per group).

plement). The shift in voltage dependence of activation in PV cells seen with intermittent burst pacing could contribute to less depolarizing current during action potential to produce PV cell APD shortening seen with intermittent burst pacing.[6]

Interestingly, when the same intermittent burst pacing protocol was applied to right atrial tissues, there were no significant changes in peak I_{Ca} density in sham and intermittent burst pacing cells (Figures 6A and 6C) or differences in voltage dependence of activation (Figure 6E), inactivation, or recovery from inactivation between sham and intermittent burst pacing in both CS and SN cells (Tables S7 and S9, Data Supplement). Therefore, unlike PV region, Ca^{2+} channels of right atrial CS cells are not remodeled by intermittent burst pacing.

L-type Ca^{2+} channel subunit mRNA expression

The L-type Ca^{2+} channel is composed of the pore-forming α_1C ($Ca_V1.2$) or α_1D ($Ca_V1.3$) subunits[19–21] and auxiliary subunits $\alpha_2\delta$ and $Ca\beta$s.[22] The effects of intermittent burst pacing on the mRNA expression levels of these subunits were determined in this study. The mRNA expression levels of L-type Ca^{2+} channel subunits $Ca_V1.2$, $Ca_V1.3$, α-2/δ, and β-2a were unaffected by 3-hour intermittent burst pacing in both PV and Bachmann bundle regions (Figure S2, Data Supplement). However, in CS region, mRNA expression levels of L-type Ca^{2+} channel subunits $Ca_V1.2$, and α-2/δ were significantly reduced by intermittent burst pacing (Figure 7). This reduction of mRNA did not yet lead to alteration in peak density of Ca^{2+} currents in CS (Figure 6). $Ca\beta$-2a was unaffected by the 3-hour intermittent burst pacing in CS regions.

Discussion

In the present study, we found differential remodeling of I_{to} and I_{Ca} in response to intermittent burst pacing in vascular sleeve cells of rabbit PV and CS. I_{to} was upregulated by intermittent burst pacing in CS cells but not in PV cells. Voltage dependence of activation of Ca^{2+} currents was shifted in a depolarizing direction by intermittent burst pacing in PV cells but not in CS cells. Interestingly, most electrophysiologic differences did not correspond to measured differences in ion channel subunit mRNA expression in intermittent burst pacing cells. It is possible that protein levels changed or remained constant, but for other posttranslational reasons, these proteins had altered subcellular localization or modified subunit interaction.

Differences in I_{to}

Chen et al[23] and Cha et al[24] reported that I_{to} in PV cells is downregulated after a 6- to 8-week period of rapid atrial pacing (≥400 bpm). In the present study, we found that I_{to} in PV cells was not affected by 3-hour intermittent burst pacing, and mRNA levels of various subunits of I_{to} were unchanged by the intermittent burst pacing protocol. Our findings are consistent with those of Bosch et al,[9] who showed that I_{to} density in rabbit atrial cells remained unaltered after the more prolonged, 12-hour rapid atrial pacing (600 bpm) protocol. From our data, it appears that the shorter intermittent pacing protocol we used is not sufficient to result in I_{to} remodeling of PV cells. However, PV cells APD still shortened.[6]

To our knowledge, no data are available on the effects of intermittent rapid atrial pacing on I_{to} in CS cells. Our results suggest that, in contrast to the findings in PV cells, I_{to} density was significantly increased in CS cells, although

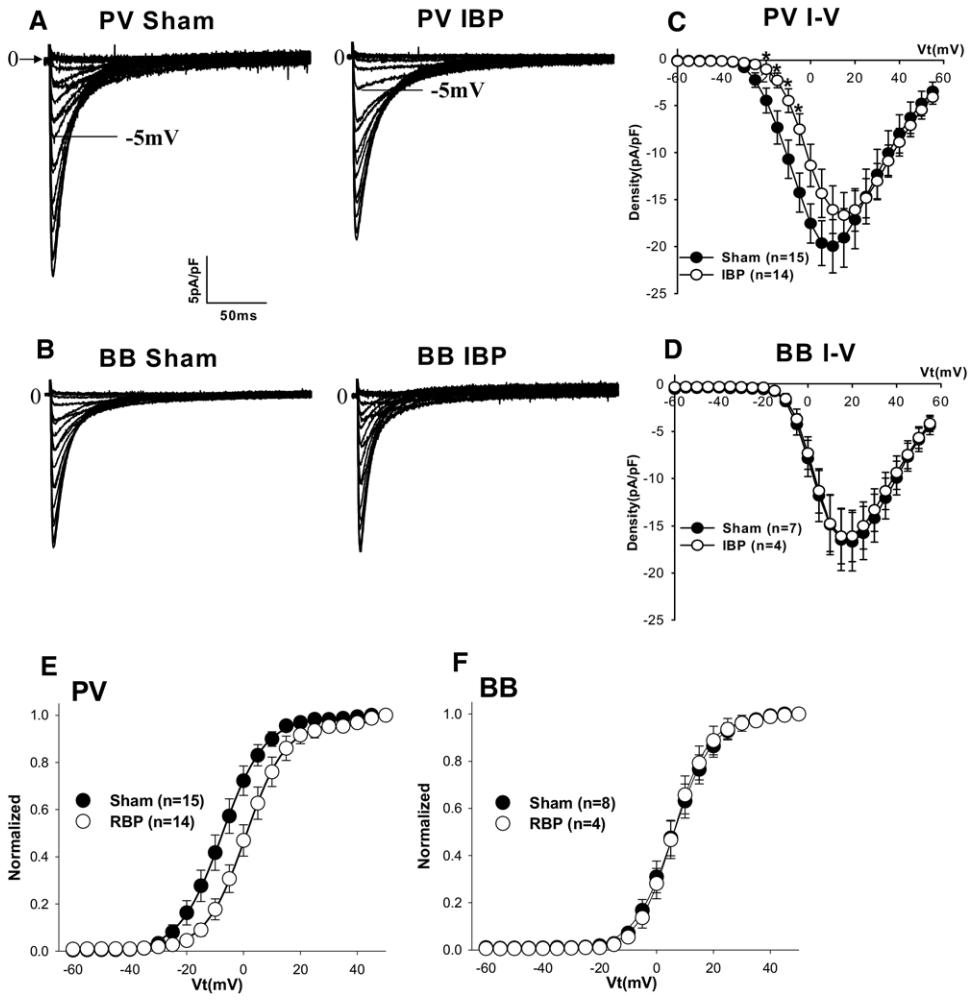

Figure 5 **A, B:** Original tracings of I_{Ca} in sham and intermittent burst pacing (IBP) cells from pulmonary vein (PV; **A**) and Bachmann bundle (BB; **B**) regions under conditions of these experiments: Ca^{2+} 3 mM; BAPTA 10 mM; holding voltage −70 mV to various test voltages from −60 to +55 mV. *Arrows* indicate zero current. **C, D:** Average peak I_{Ca} density-voltage relations in PV and BB. Data were collected at similar times (10 minutes) after whole-cell membrane rupture. Data are given as mean ± SE. **E, F:** Activation curves of PV and BB cells. $V_{0.5}$ and slope factor k were calculated from the current–voltage protocol and E_{Rev} and are shown in Table S6. IBP PV cells showed significant positive shifts of activation curves vs sham PV cells. All data were collected at the same time (10 minutes) after whole cell rupture.

there was no corresponding increment in mRNA levels of I_{to} subunits studied. In contrast, mRNA levels of $K_V4.3$ and $K_V1.4$ were significantly reduced with intermittent burst pacing. Thus, again we can infer that posttranscriptional alterations of I_{to} subunits (e.g., ionic channel subunit traffic, accessory subunit processing, and pore-forming subunit regulation) importantly contributed to the upregulated I_{to} density observed with intermittent burst pacing in CS cells.

$K_V1.5$ underlies atrial-specific I_{Kur} in humans, dogs, and rats.[14,15] In chronic human AF, $K_V1.5$ protein levels were significantly decreased.[8] In the chronic dog AF model, I_{Kur} density remained stable between 0 and 42 days of rapid pacing.[25] In short-term sustained rapid pacing in rat, $K_V1.5$ mRNA and protein transiently increased in the atrial membrane fraction during a 2- to 8-hour persistent pacing period.[26] To our knowledge, no data are available on $K_V1.5$ expression and function in rabbit atria. In the present study, using C9356 (a selective $K_V1.5$ blocker[14]), we showed that C9356-sensitive currents were stable in response to intermittent burst pacing in both CS and PV cells, although mRNA levels of $K_V1.5$ were significantly decreased by intermittent burst pacing in the same regions. Thus, it is possible that 3-hour intermittent burst pacing only interrupts mRNA expression levels for $K_V1.5$ but does not alter protein abundance over this time period. Again, stable currents occurred in the presence of measurable mRNA level changes with the 3-hour intermittent burst pacing protocol.

Differences in I_{Ca}

Chen et al[23] and Cha et al[24] reported reduced I_{Ca} density in PV cells after a 6- to 8-week period of sustained rapid atrial pacing (400 bpm) in dogs, with no changes in kinetics of Ca^{2+} currents between rapid atrial pacing and nonpacing controls. Bosch et al[9] showed that after 6 hour of sustained rapid atrial pacing, I_{Ca} densities were not significantly different from sham in rabbit atrial cells, yet a reduction in $Ca_V\beta2$ subunit mRNA levels had occurred. In the present study, we demonstrated that in PV cells, Ca^{2+} current I–V

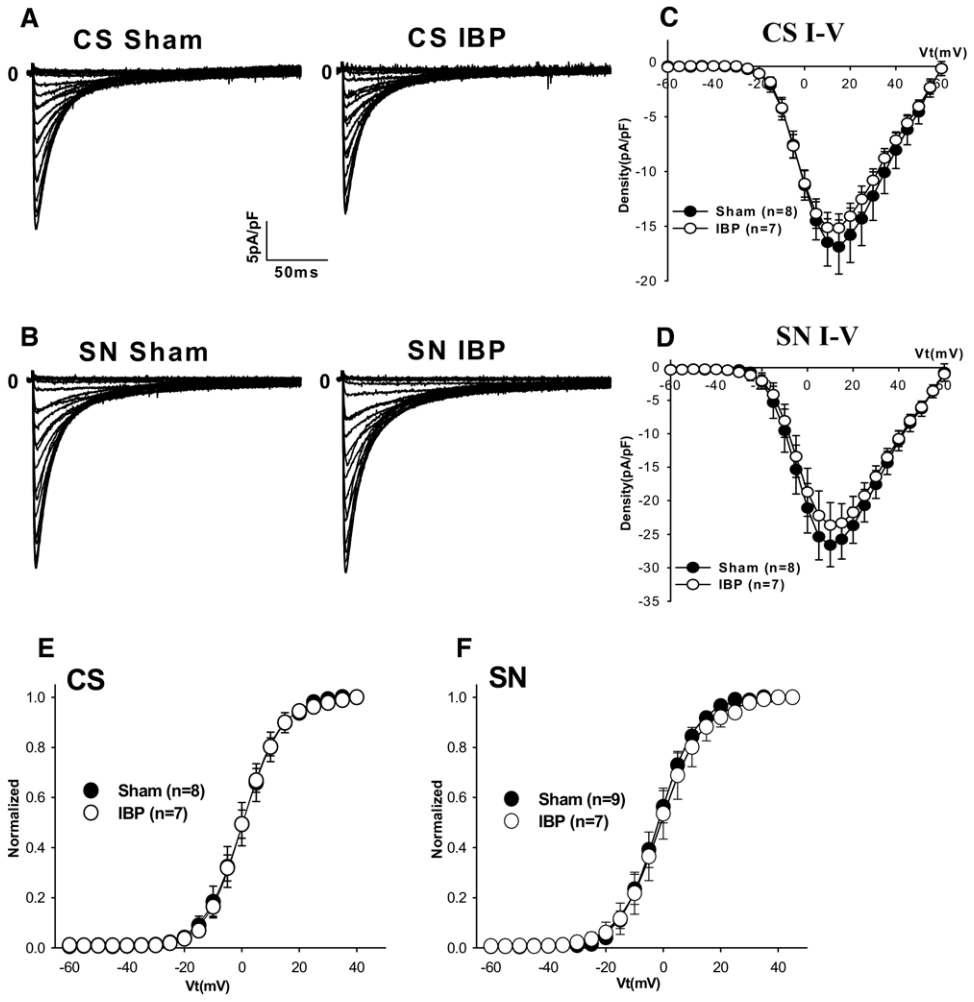

Figure 6 **A, B:** Original tracings of I_{Ca} in sham and intermittent burst pacing (IBP) cells from coronary sinus (CS; **A**) and sinus node (SN; **B**) regions under conditions of these experiments: Ca^{2+} 3 mM; BAPTA 10 mM; holding voltage −70 mV to various test voltages from −60 to +55 mV. *Arrows* indicate zero current. **C, D:** Average peak I_{Ca} density-voltage relations in CS and SN. Data were collected at similar times (10 minutes) after whole-cell membrane rupture. Data are given as mean±SE. **E, F:** Activation curves of CS and SN cells. $V_{0.5}$ and slope factor k were calculated from the current–voltage protocol and E_{Rev}, and mean values are shown in Table S7. There are no significant shifts of activation curves between sham and IBP in CS and SN cells. All data were collected at the same time (10 minutes) after whole-cell rupture.

curve was shifted in a depolarized direction after intermittent burst pacing, although there was no difference in peak I_{Ca} density between sham and intermittent burst pacing cells. This shift in activation relation in PV cells seen with intermittent burst pacing could account for less depolarizing Ca^{2+} current during an action potential.[6] In cardiac muscle, L-type Ca^{2+} channels are primarily encoded by the α_{1C} gene ($Ca_V1.2$), and in some cell types there is a possible contribution by α_{1D} ($Ca_V1.3$).[22] Ca^{2+} currents of atrial cells from $Ca_V1.3$-deficient mice show a significant depolarizing

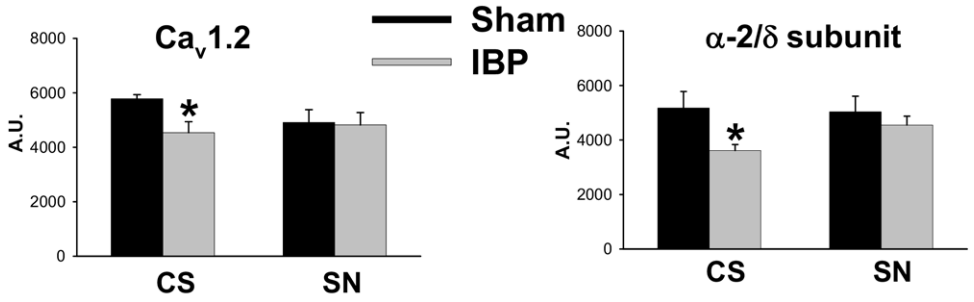

Figure 7 Effects of intermittent burst pacing (IBP) on mRNA levels of $Ca_V1.2$ and α-2/δ subunits in coronary sinus (CS) and sinus node (SN). A.U. is a relative arbitrary unit. Data are given as mean ± SE (n = 7 rabbits per group).

shift in voltage dependence of activation with no peak density change,[27] similar to our findings in intermittent burst pacing PV cells, suggesting that intermittent burst pacing may have selectively reduced the α_{1D} isoform contribution to PV Ca^{2+} currents. However, our mRNA data did not reveal a reduction of α_{1D} or $Ca_V\beta 2$ levels with intermittent burst pacing in the PV region. Importantly and unlike PV cells, Ca^{2+} currents in CS cells are not remodeled by intermittent burst pacing, although mRNA levels of α_{1C} and α_{1D} decreased with 3-hour intermittent burst pacing.

Study limitations

This study used an intermittent burst pacing protocol to establish the early effects of pacing on ionic currents and steady-state mRNA levels in four different regions of rabbit atria. We did not complete quantitative protein studies in these cells/tissues because of the very small regions involved and the lack of robust antibodies for the various subunits. Furthermore, the findings in the four regions studied do not mean that intermittent burst pacing did not cause ionic changes in other regions not studied.

Nevertheless, we found that there are differences in ionic channel remodeling in response to intermittent burst pacing between PV and CS cells. The upregulated I_{to} in intermittent burst pacing CS cells may account for the APD shortening observed in intermittent burst pacing CS region.[6] The significant depolarization shift in voltage-dependent activation of I_{Ca} appears to contribute to the APD shortening seen with intermittent burst pacing in the PV cell region.[6] Further, intermittent burst pacing did not affect I_{to} and I_{Ca} in both Bachmann bundle and SN, consistent with cell APD data.[6] The region-specific early electrical remodeling in response to intermittent burst pacing in PV and CS cells differs, and this knowledge is helpful in understanding the differences in substrates between left atrial PV cells and right atrial CS cells.

Appendix

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hrthm.2006.12.032.

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