

Changes in Atrial Effective Refractory Period and I_{KACH} After Vagal Stimulation Plus Rapid Pacing in the Pulmonary Vein

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Introduction and objectives. Recent studies have shown that rapid atrial pacing causes atrial electrical remodeling. However, the influence of the vagus nerve on atrial electrical remodeling is not clear.

Methods. This study involved 24 dogs divided into 3 groups. In the control group, the inducibility of atrial fibrillation (AF) during vagal stimulation (VS_1) was investigated. In the pacing group, the atrial effective refractory period (AERP) was determined before and after pacing in the left superior pulmonary vein (LSPV). In the vagal stimulation (VS) plus pacing group, the LSPV was subjected to rapid electrical pacing after vagal stimulation (VS_2), and the AERP was measured both before VS_2 and after pacing. The I_{KACH} density was measured in LSPV and atrial myocardial cells in the 3 groups using the patchclamp technique.

Results. The duration of induced AF was greater in the pacing group than in the control or VS-plus-pacing group. In the pacing group, the AERP was markedly shortened and the AERP dispersion (dAERP) was significantly increased ($P < .05$). However, there was no significant change in AERP in the VS-plus-pacing group, though the dAERP increased significantly ($P < .05$). The I_{KACH} density was increased in LSPV and atrial myocardial cells after pacing. However, there was no significant change in I_{KACH} density after VS_2 plus pacing.

Conclusions. Although shortening of the AERP may play a fundamental role, it is not in itself responsible for cholinergically induced AF. Rapid pacing in the LSPV increased the I_{KACH} . However, VS before rapid pacing partly protected the atria against electrical remodeling.

Key words: Vagus nerve. Electrical remodeling. Atrial fibrillation. Pacing. Dogs.

Cambios del periodo refractario efectivo auricular y de la I_{KACH} tras estimulación vagal más estimulación eléctrica rápida en venas pulmonares

Introducción y objetivos. Estudios recientes han demostrado que la estimulación auricular rápida causa un remodelado eléctrico auricular. Sin embargo, no se ha aclarado el efecto del nervio vago en el remodelado eléctrico auricular.

Métodos. Se dividió un total de 24 perros en 3 grupos. En el grupo control, se estudió la inducibilidad de fibrilación auricular (FA) durante la estimulación vagal (EV_1). En el grupo de estimulación eléctrica (EE), se determinó el periodo refractario efectivo auricular (PREA) antes y después de la estimulación en la vena pulmonar superior izquierda (VPSI). En el grupo de $EV + EE$, tras la estimulación vagal (EV_2), se aplicó EE rápida en la VPSI. Se determinó el PREA antes de la EV_2 y después de la EE. Se determinaron las densidades de I_{KACH} en las células de VPSI y de miocardio auricular mediante la técnica de patch clamp en los 3 grupos.

Resultados. La duración de la FA inducida en el grupo de EE fue superior a la de los grupos de control y de $EV + EE$. En el grupo de EE, el PREA presentó un acortamiento marcado y la dispersión del PREA (dPREA) aumentó de forma significativa ($p < 0,05$). Sin embargo, en el grupo de $EV+EE$ el PREA no se modificó de forma significativa, mientras que la dPREA aumentó significativamente ($p < 0,05$). Las densidades de I_{KACH} aumentaron en las células de VPSI y del miocardio auricular tras la aplicación de la estimulación eléctrica. Sin embargo, no hubo diferencias significativas en las densidades de I_{KACH} tras la EV_2 más EE.

Conclusiones. La disminución del PREA puede ser fundamental, pero no es de por sí causa de la inducción colinérgica de la FA. La estimulación rápida en la VPSI aumentó la I_{KACH} . Sin embargo, la EV antes de la EE rápida protege en parte a las aurículas contra el remodelado eléctrico.

Palabras clave: Vago. Remodelado eléctrico. Fibrilación auricular. Estimulación eléctrica. Perros.

SEE EDITORIAL ON PAGES 729-32

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Received December 2, 2008.

Accepted for publication February 18, 2009.

ABBREVIATIONS

AERP: atrial effective refractory period.
 AF: atrial fibrillation.
 APD: action potential duration.
 dAERP: dispersion of AERP.
 LA: left atrium.
 LSPV: left superior pulmonary vein.
 RA: right atrium.
 VS: vagal stimulation.

INTRODUCTION

Recent experimental and clinical studies have established the role of the autonomic nervous system, particularly the parasympathetic nervous system, in the pathogenesis of atrial fibrillation (AF).¹⁻⁴ However, few reports have focused on the relationship between parasympathetic tone and recovery from electrical remodeling. Blaauw et al⁵ reported that high vagal tone was associated with a short atrial effective refractory period (AERP) after rapid pacing and that there was a prolonged recovery from remodeling in goats. Miyauchi et al⁶ demonstrated that blockage of the parasympathetic system may facilitate early recovery from electrical remodeling associated with short-term rapid pacing. However, in another study, Takei et al⁷ showed that VS prior to rapid pacing prevented electrical remodeling. It is widely accepted that VS is mediated by release of acetylcholine-regulated receptors and activates the atrial acetylcholine-regulated potassium current ($I_{K_{ACh}}$); consequently there is shortening of the AERP, the atrial action potential duration (APD), and that enhances the dispersion of the AERP (dAERP), inducing AF.^{8,9} The changes in atrial electrical properties (electrical remodeling) are associated with the activation of $I_{K_{ACh}}$. Thus, we hypothesize that the effect of vagal tone on atrial electrical remodeling is related to the densities of $I_{K_{ACh}}$. To study the mechanism of the effect of vagal tone on electrical remodeling, we investigated the changes in the AERP and $I_{K_{ACh}}$ after VS and rapid pacing in left superior pulmonary vein (LSPV) and discuss the relationship between parasympathetic tone and recovery after electrical remodeling.

METHODS

Experimental Animals

Twenty-four dogs, weighing 15 to 22 kg (mean, 20 [3] kg) were used in the study. The animals were anesthetized via the abdominal route with pentobarbital sodium (30 mg/kg body

weight) and ventilated with room air. After a median sternotomy, the heart was exposed in a pericardial cradle. The bilateral cervical vagal trunks were then severed to impede the arrival of all tonic neural activity to the heart. Continuous electrocardiographic monitoring was carried out using leads II and aVF.

Electrophysiological Measurements

The 3 custom-built electrode probes were applied with an electrode operator (UNM-1, Japan) to the right and left atrial epicardial surfaces, and to the LSPV. Reference electrodes were fixed to the chest wall. The AERP was determined by a LEAD-2000B instrument (Sichuan, China). Electrode probe electrograms were filtered at 30-500 Hz. Electrocardiographic filter settings ranged from 0.1 to 250 Hz. The S_1 - S_2 intervals were decreased from 150 ms to refractoriness, initially by decrements of 10 ms (S_1 : S_2 =8:1). As the S_1 - S_2 intervals approached the AERP, decrements were reduced to 5 ms. An extra stimulus (S_2) was added late in atrial diastole, and the interval between S_1 and S_2 was reduced in 5-ms steps until there was no propagated atrial response. The longest S_1 S_2 coupling interval that failed to result in a propagated atrial response was taken as the local AERP.

Experiment Protocol

Twenty-four dogs, divided into 3 groups of 8 each, were used for the study as follows: control group, pacing group and VS-plus-pacing group.

In the control group, VS was achieved by introducing silver wires into the right cranial end of the vagosympathetic trunk towards the canine heart. Electrical stimulation was then delivered at a frequency of 20 Hz, in pulses of 0.2-ms duration (electrophysiological stimulator SEN-7103, Japan). The voltage chosen for VS was 5 V above the voltage at which a sinus arrest lasting over 2 seconds was achieved. This stimulation protocol was referred to as VS_1 . The inducibility of AF was assessed during the same period. When AF was induced, VS_1 was concluded. If after 15 seconds of VS_1 , AF was not induced, electrical stimulation was also discontinued (Figure 1).

In the pacing group, 8 dogs were subjected to LSPV pacing at 500 beats/minute for 4 hours. The AERP was measured in right atrium (RA), left atrium (LA), and LSPV both before and after pacing, after which, VS_1 was recorded and the inducibility of AF was again measured.

In the VS-plus-pacing group, silver wires were introduced into the right vagosympathetic trunks towards the canine hearts. After determining the

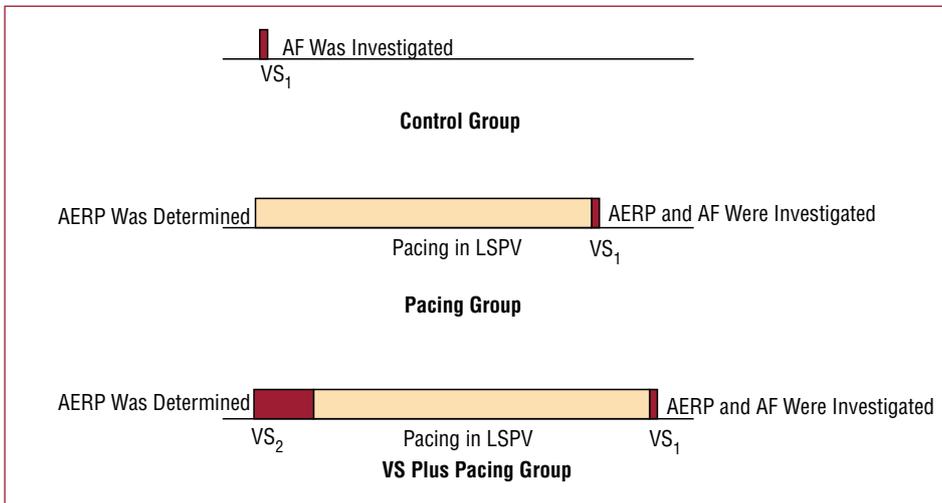


Figure 1. Presentation of the experimental protocol. AERP, atrial effective refractory period; AF, atrial fibrillation; LSPV, left superior pulmonary vein; VS, vagal stimulation.

AERP, electrical stimulation was delivered at a frequency of 5 Hz, in pulses of 0.2 ms duration and at a voltage of 5-10 V for 30 minutes. This stimulation protocol was referred to as VS_2 . We selected a lower stimulation frequency for VS_2 to avoid second or third-degree atrioventricular block and permitted atrial pacing to be conducted to the ventricle during VS_2 . The LSPV was then subjected to rapid pacing at 500 beats/min for 4 hours. After cessation of pacing, the AERP was measured and AF inducibility was assessed again during VS_1 . The dAERP was calculated by determining the difference between the highest and lowest AERP from 3 AERP recorded at the same time.

Patch-clamp Techniques

After electrophysiological measurements, the canine hearts were excised and immersed in normal saline at 0°C. The tissues were dissected from the RA, LA, and LSPV were immediately kept in 3 separate beakers containing Ca^{+} -free Tyrode solution (30 mL) containing 136 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 0.33 mM NaH_2PO_4 , 10 mM glucose and 5 mM HEPES (pH, 7.4) with 100% O_2 at 37°C. Single atrial myocytes were obtained by the dispersion method as previously described.¹⁰ Overall, it took 1 hour to isolate the cells. Many viable cells were isolated from each of the 3 regions but only 1 to 2 cells were used for the patch-clamp technique, which took about 2 hours.

The whole-cell configuration of the patch-clamp technique was used in this study. The isolated cells were perfused with the Tyrode solution containing 136 mM NaCl, 5.4 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES (pH, 7.4). The pipette solution was composed of 110 mM

potassium aspartate, 20 mM KCl, 1 mM $MgCl_2$, 5 mM Mg-ATP, 0.1 mM GTP, 10 mM EGTA, 5 mM phosphocreatine, 10 mM HEPES, and the pH was adjusted to 7.3 with KOH. Command pulses were generated by a converter controlled by Pulse/Pulsefit software (Heka Instruments, Germany). Junction potentials were set to zero before the formation of the membrane-pipette seal in the Tyrode solution. The capacitance and series resistance were both electrically compensated to minimize the duration of the capacitive surge on the current recording and the voltage drop across the clamped cell membrane. Cells with changing leak current (indicated by changes of more than 10 pA in the holding current at -50 mV) were rejected. Experiments were conducted at 32 [1]°C.

To record I_{KACH} , other subtypes of muscarinic cholinergic receptors were inhibited using the subtype-selective antagonists pirenzepine (100 nM, an M_1 blocker), 4-DAMP (2 nM, an M_3 inhibitor) and tropicamide (200 nM, an M_4 inhibitor). This is the only I_{KACH} change marked by the authors. I_{KACH} was induced by 1 μM ACh and recordings of I_{KACH} were generally conducted with dofetilide (1 μM) and chromanol 293B (20 μM) in the bathing solution to block IKr and IKs. Contamination by sodium current was prevented by holding the cell at -50 mV. Cadmium chloride (200 μM) was used to inhibit the Ca^{2+} current as well as the Ca^{2+} -activated chloride current. The ATP-sensitive K^+ current, if present, was suppressed by glyburide (10 μM) in the perfusate and by 5 mM Mg-ATP in the pipette.¹¹ I_{KACH} was induced by ACh (1 μM) in the bathing solution and defined as the atropine (1 μM)-sensitive current to rule out contamination from the background inward rectifier K^+ current (I_{K1}).

TABLE 1. Characteristics of Atrial Fibrillation Induction

	Control	Pacing	VS+Pacing
Number of animals	8	8	8
Number sustained AF (>5 s)	0	8*	0
Mean duration of AF, s	0.625	14*	1.125
Longest duration of AF, s	5	17*	5

*Pacing versus control and VS+pacing, $P<.01$
AF indicates atrial fibrillation.

TABLE 2. Changes in Atrial Effective Refractory Period Before and After Vagal Stimulation in the Pacing and Vagal Stimulation-Plus-Pacing Groups

	AERP, ms		
	LSPV	LA	RA
Pacing group			
Before pacing, mean (SEM), ms	133 (6)	142 (7)	141 (7)
After pacing, mean (SEM), ms	101 (8) ^a	119 (10) ^a	114 (9) ^a
VS+pacing group			
Before VS ₂ +pacing, mean (SEM), ms	136 (6)	143 (8)	140 (7)
After VS ₂ +pacing, mean (SEM), ms	124 (11)	139 (12)	139 (12)

^aBefore versus after VS₁, $P<.01$; before versus after pacing, $P<.05$.

Table 2 shows that AERP was shortened at all sites after VS or pacing in pacing group. However, there was no significant difference in AERP before and after pacing in VS+pacing group. LSPV pacing was applied at 8.33 Hz, VS₁ at 20 Hz, and VS₂ at 5 Hz.

AERP indicates atrial effective refractory period; LA, left atrium; LSPV, left superior pulmonary vein; RA, right atrium; SEM, standard error of the mean; VS, vagal stimulation.

Statistical Analysis

Values are expressed as means plus or minus the standard error of the mean. The SPSS statistical software package was used for analysis. The statistical comparisons were performed with ANOVA. The comparisons of paired and of unpaired data were carried out using the Student *t* test and incidences of AF were compared by Fisher's exact test. Statistical significance was assumed if *P* values were less than .05.

RESULTS

Induction of Atrial Fibrillation

In the control group, AF was induced in 1 of the 8 animals. The duration of AF was 5 seconds. In the pacing group, AF was induced in all 8 animals and its duration was over 10 seconds. In the VS-plus-pacing group, AF was induced in 2 animals. The duration of AF was 4 seconds in 1 animal and 5 seconds in the other. The incidence of induced AF was higher and its duration longer in the pacing group than in the control and the VS-plus-pacing groups ($P<.05$); however, there were no significant differences between the control group and the VS-plus-pacing group (Table 1).

Changes in the Atrial Effective Refractory Period and in the Dispersion of the Atrial Effective Refractory Period

In the pacing groups, the AERP was markedly shorter at all the sites and the dAERP was significantly increased (11 [3] ms vs 32 [5] ms; $P<.05$), respectively. However, in the VS-plus-pacing group, the AERP was not significantly changed after pacing, whereas the dAERP also increased significantly (10 [3] ms vs 30 [5] ms; $P<.05$) (Tables 2 and 3).

Correlation Between Pacing and I_{KACH} Density

The amplitude of I_{KACH} was measured as an average of the currents at the end of the two-second voltage steps after the onset of these voltage steps. As illustrated, in the control group, the densities of I_{KACH} were substantially lower in the LSPV cells than those observed in the atrial myocytes at all the potentials tested. Furthermore, the I_{KACH} densities were lower in the right atrial myocytes than in the left atrial myocytes (LA, RA vs LSPV: -14 [0.58], -10 [0.63] vs -7 [0.42] pA/pF; $P<.05$). In the pacing group, the densities of I_{KACH} were increased at all sites (LSPV: -17 [0.61] vs -14 [0.58] pA/pF; LA: -13 [0.57] vs -10 [0.63] pA/pF; RA: -11 [0.53] vs -7 [0.42]

TABLE 3. Changes in Dispersion of Atrial Effective Refractory Period Before and After Pacing (Vagal Stimulation1) in the Pacing and Vagal Stimulation-Plus-Pacing Groups

	dAERP, ms	
	Before Pacing (or VS)	After Pacing (or VS)
Pacing group, mean (SEM), ms	11 (3)	32 (5)*
VS+pacing group, mean (SEM), ms	10 (3)	30 (5)*

*Before versus after VS, $P < .05$.

Table 3 shows that dAERP increased significantly after pacing or VS in the pacing and VS+pacing groups. dAERP indicates dispersion of atrial effective refractory period; SEM, standard error of the mean; VS, vagal stimulation.

pA/pF; $P < .05$). However, in the VS-plus-pacing group, the I_{KACH} densities showed a decreasing trend in LA, RA, and LSPV, but this did not attain statistical significance (-12 [0.42] vs -14 [0.58] pA/pF, -9 [0.51] vs -10 [0.63] pA/pF; -6 [0.37] vs -7 [0.42] pA/pF; $P > .05$) (Figures 2 and 3).

DISCUSSION

The results of the present study show that: a) prior to rapid pacing, VS can inhibit the vulnerability to AF, and b) rapid burst pacing in LSPV increases the densities of I_{KACH} in the atrium and LSPV, while VS prior to pacing inhibits the changes in I_{KACH} . These results suggest that the effect of rapid pacing on atrial electrical remodeling is related to an increase in the I_{KACH} .

Recent studies have suggested that the vagal nerve plays an important role in the development of and recovery from atrial electrical remodeling associated with rapid pacing.^{5,7} This study indicates that an increase in vagal tone together with electrical remodeling might act synergistically to shorten the refractory period and promote AF. Similarly, Miyauchi et al⁶ showed that parasympathetic blockade with atropine promoted recovery from atrial electrical remodeling induced by short-term atrial pacing in humans. However, Takei et al⁷ demonstrated that vagal stimulation prior to rapid atrial pacing prevented electrical remodeling. Perhaps the different findings indicate that the vagal tone has different effects on the electrical remodeling before and after rapid atrial pacing.

Data from other studies have demonstrated a marked shortening of the APD and formation of early after-depolarizations in superfused pulmonary vein sleeves when exposed to acetylcholine and norepinephrine or with local electrical stimulation.^{12,13} Rapid activations within the pulmonary veins are important in the mechanisms of AF. The LSPV is a frequent source of these rapid activations during AF.¹⁴ To investigate the effect of VS on electrical remodeling before rapid LSPV pacing, we observed different changes in the AERP

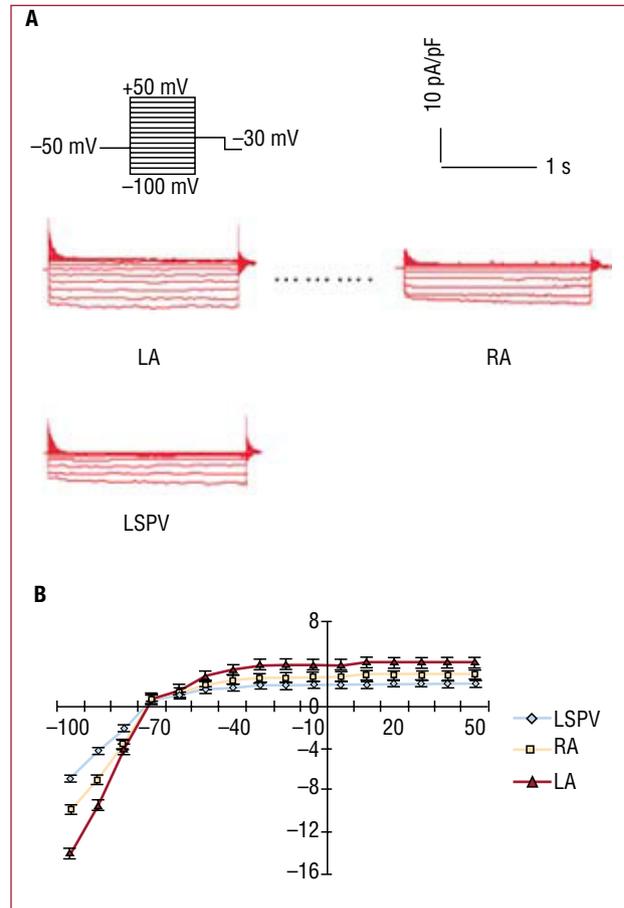


Figure 2. A: comparison of I_{KACH} in LA, RA, LSPV in control group. Two-second voltage steps were delivered to elicit I_{KACH} with potentials ranging from -100 mV to +50 mV. The same voltage protocols were applied to the subsequent figures. I_{KACH} was activated in the presence of 1 μ M ACh. The dashed line indicates zero current level. B: current density-voltage relationships of the currents (n=6 cells for LA, n=6 cells for RA, n=7 cells for LSPV). I_{KACH} was measured at the end of the 2-s pulses. I_{KACH} densities in LA and RA are higher than that of LSPV. Furthermore, density of I_{KACH} is higher in LA than in RA. ACh, acetylcholine; LA, left atrium; LSPV, left superior pulmonary vein; RA, right atrium.

after VS plus rapid LSPV pacing. We found that after pacing without VS, there was a sharp decrease in the AERP and a significantly increased dAERP. However, after VS plus pacing, the AERP did not

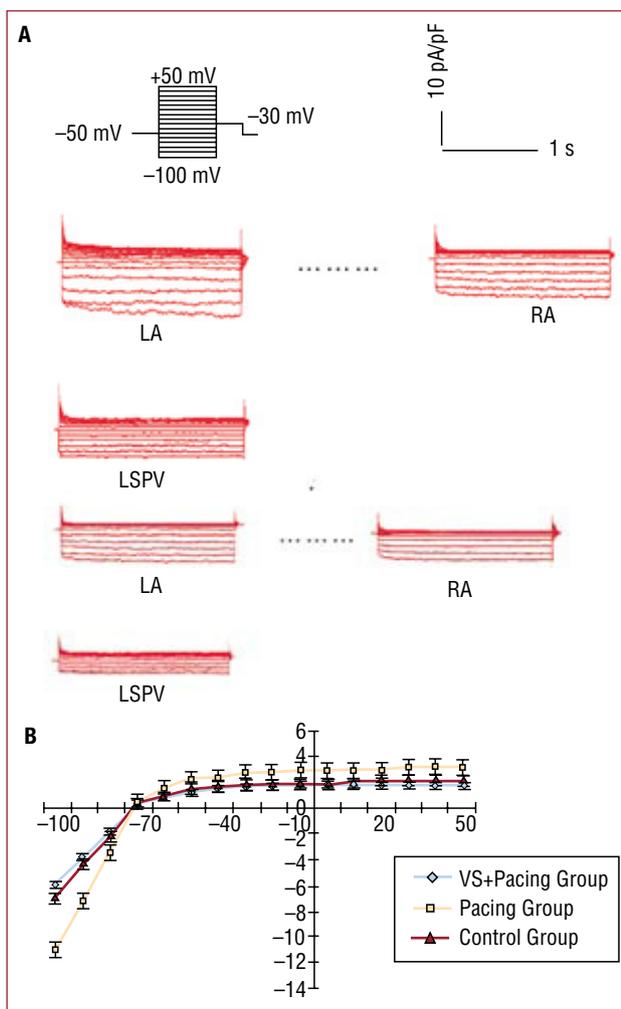


Figure 3. A: comparison of I_{KACH} in LA, RA, and PV between pacing group and VS+pacing group. Two-second voltage steps were delivered to elicit I_{KACH} with potentials ranging from -100 mV to +50 mV. The same voltage protocols were applied to the subsequent figures. I_{KACH} was activated in the presence of 1 μ M ACh. The dashed line indicates zero current level. B: current density-voltage relationships of the currents in LSPV (n=7 cells for control group, n=6 cells for LSPV pacing group, n=7 cells for VS plus LSPV pacing group). I_{KACH} was measured at the end of the 2-s pulses. In pacing group, I_{KACH} density is higher than that in VS group. However, there were no significant differences in VS group and VS+pacing group. ACh, acetylcholine; LA, left atrium; LSPV, left superior pulmonary vein; PV, pulmonary vein; RA, right atrium; VS, vagal stimulation.

change significantly, while the dAERP increased significantly. Induced AF and AF duration in the pacing group were greater than in the control group and the VS-plus-pacing group; however, there was no significant difference between the control group and the VS-plus-pacing group. The results showed that VS plus rapid LSPV pacing could protect against a decrease in the AERP, but could not wholly protect the atrium from electrical remodeling. It is well known that heterogeneity

in atrial innervation contributes to the ability of the VS to initiate reentrant AF by increasing the dispersion of refractoriness within the atrium.¹⁵⁻¹⁷ In the present study, the results showed that increases in the dAERP alone were not enough to induce AF but, rather, the decrease in the AERP, was the basis of the initiation of AF.

Several studies have demonstrated different distributions of I_{KACH} in the atrium and pulmonary veins.^{14,18-20} Chronic atrial tachycardia in the range of that of AF produces important alterations in ion channel function (reduced densities of transient outward K^+ current I_{to} , L-type Ca^{2+} current I_{Ca} , and Na^+ current I_{Na}) that result in a functional substrate that supports the maintenance of AF.²¹⁻²⁴ In chronic human AF, Dobrev et al²⁵ showed that down regulation of I_{KACH} attenuates the muscarinic receptor-mediated shortening of APD. Furthermore, in their other study, they demonstrate that larger basal inward rectifier K^+ current in chronic AF consists of increased I_{K1} activity and constitutively active I_{KACH} .²⁶ These results showed that the shortening of the AERP due to electrical remodeling was counteracted by down regulation of I_{KACH} .

In the present study, we observed the densities of I_{KACH} at different sites and under different conditions. The results showed that, after pacing, the densities of I_{KACH} were increased in LSPV, LA and RA. The mechanisms by which the densities of I_{KACH} change with rapid atrial pacing or sustained AF are unknown. Dobrev et al. suggested that atrial myocytes adapt to a chronically high rate by downregulating I_{KACH} to counteract the shortening of the AERP due to electrical remodeling. However, our data showed that, after 4 hours of rapid pacing, densities of I_{KACH} were increased. After VS plus pacing, we observed that there were no differences in I_{KACH} between sinus rhythm and after VS prior to pacing. This remodeling of I_{KACH} may explain why VS plus pacing protected the atrium from atrial electrical remodeling. In our previous study, we found that rapid pulmonary vein pacing induced a decrease in $I_{Ca,L}$ and I_{to} densities.²⁷ To our knowledge, the essential elements required for this process are currently unknown. A recent study showed that I_{to} in rabbit atrium is depressed after short-time rapid atrial pacing but recovers after a longer pacing period.²⁸ The time course of I_{KACH} remodeling when oscillations are produced should be further investigated.

Limitations of the Study

Pentobarbital is known to prolong the AERP as compared to the unanesthetized state and it affects sympathetic and parasympathetic tone,

which may be a limitation in the present study. All the dogs were self-controlled and received the same dose as well as the same kind of anesthetic, and pentobarbital sodium has little effect on the autonomic nerve as compared with VS. In our study, we observed the changes in the AERP and I_{KACH} after only 4 hours of pacing. The effect of long-term pacing on I_{KACH} may yield different results and should be further investigated.

Furthermore, we did not examine the activity of I_{KACH} after VS (5 Hz frequency, with a 0.2 ms pulse duration and at a voltage of 5-10 V) for 30 minutes, and continuous VS during rapid pacing might have yielded different results. Finally, this study addressed neither the question of the time course necessary to influence I_{KACH} nor the hemodynamic variables during rapid pacing. However, there were no significant differences in peripheral edema or skin temperature between the 2 groups. Future studies should investigate whether the vagal tone influences I_{KACH} and the effect of hemodynamic variables on vagal tone.

CONCLUSION

Decreased AERP may be fundamental, but is not the only cause for the cholinergic induction of AF. Rapid LSPV pacing can increase I_{KACH} ; however, VS prior to rapid pacing partially protects the atria from electrical remodeling.

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