Effect of Bepridil in Atrial Fibrillation Inducibility Facilitated by Vagal Nerve Stimulation

– Prevention of Vagal Nerve Activation-Induced Shortening of the Atrial Action Potential Duration –

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Background: Because bepridil blocks multiple myocardial ionic channels, including the muscarinic acetylcholine receptor-operated potassium current ($I_{K_{ACh}}$), bepridil is expected to suppress atrial fibrillation (AF) mediated by vagal nerve stimulation (VNS).

Methods and Results: The therapeutic effects of bepridil were studied with a special focus on heart rate variability (HRV) in a canine model of AF. During VNS, AF was induced in 9 of 9 experiments before, vs 3 of 9 experiments after administration of bepridil ($P < 0.01$). During 350 ms atrial pacing, VNS shortened the right and left atrial monophasic action potentials at 90% repolarization (MAP90) by $-31 \pm 8\%$ and $-22 \pm 12\%$, respectively, vs $-10 \pm 13\%$ and $-6 \pm 8\%$, respectively, after bepridil ($P < 0.01$, $N = 9$). Bepridil prolonged the sinus cycle length, although it had no significant effect on the conduction time measured at 300 ms pacing. Statistically insignificant change was observed in the VNS-induced slowing of the sinus cycle length and in the VNS-induced increase in high frequency amplitude of HRV before (1.2 ± 0.7 to 5.3 ± 4.0 ms) vs after (1.7 ± 0.8 to 5.4 ± 2.3 ms) bepridil administration.

Conclusions: Bepridil prevented the VNS-induced shortening of atrial MAP90 and suppressed the inducibility of AF during VNS in two-thirds of the experiments. As far as this study shows, it may be possible that inhibition of $I_{K_{ACh}}$ played a part in this antifibrillatory effect. (*Circ J* 2010; **74**: 895–902)

Key Words: Atrial fibrillation; Bepridil; Muscarinic acetylcholine receptor-operated potassium current; Vagal nerve activity

Bepridil, a pharmaceutical that inhibits multiple myocardial ionic currents, including calcium, sodium and several potassium currents,^{1–3} is used to treat ventricular tachyarrhythmias and atrial fibrillation (AF).^{4,5} Although bepridil has been found to be highly effective in the treatment of AF,^{4–7} its electrophysiological properties are not fully understood.

In AF associated with structural heart disease, an arrhythmogenic substrate is created by atrial enlargement and by structural and electrophysiological remodeling of the myocardium. On the other hand, triggered activity originating from the pulmonary veins, and reentrant activation around their orifices, are important mechanisms of lone AF.^{8,9} In both instances, enhanced vagal nerve activity is proarrhythmic and facilitates the development of AF.

Because bepridil inhibits the muscarinic acetylcholine receptor-operated potassium current ($I_{K_{ACh}}$),¹⁰ it is expected to

be effective in the treatment of AF associated with vagal activity, by inhibiting the acetylcholine-induced increase in $I_{K_{ACh}}$ in the atrium. We tested this hypothesis in a previously described canine model of AF facilitated by vagal nerve stimulation (VNS).^{11–13} Besides the inducibility of AF and the electrophysiological characteristics of the atrium, we studied the antiarrhythmic effects of bepridil in AF associated with enhanced vagal nerve activity, and measured autonomic nervous activity using heart rate variability (HRV).

Methods

Animal Preparation

This study was approved by the Animal Studies Subcommittee of our Institutional Review Board, and was in compliance with the guidelines issued by the United States National Institutes of Health for the Care and Use of Laboratory Animals.

Received September 27, 2009; revised manuscript received January 15, 2010; accepted January 31, 2010; released online April 3, 2010
Time for primary review: 29 days

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ISSN-1346-9843 doi:10.1253/circj.CJ-09-0716

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Surgical Procedures

The experiments were performed in 9 beagles each weighing 9.5–10.3 kg, anesthetized with a 17.5-mg/kg i.v. bolus of sodium thiopental, intubated and artificially ventilated. To minimize the effect of anesthetics on the autonomic nervous system, a 3.0–5.0 mg·kg⁻¹·h⁻¹ dose of thiopental was infused to maintain a shallow and constant sedation. Catheters were inserted into the femoral vein to administer fluids and drugs, and into the femoral artery to monitor arterial blood pressure. Leads I, II, III and aVF of the surface electrocardiogram and blood pressure were continuously monitored. The core temperature was kept at 37°C with a thermostatically controlled thermal blanket and infrared heating system. To stimulate the vagal nerve, two 75-μm silver wire electrodes were inserted into the right cervical vagal trunk, and square pulses of 0.5–2.0 V in strength and 0.3 ms in duration were delivered at a frequency of 20 Hz.^{14,15} The heart was exposed via midline sternotomy. A model 1675P Franz catheter (Boston Scientific, Natick, MA, USA) was inserted into the left pulmonary vein and advanced retrogradely to the lateral side of the left atrium near the mitral valve, to record left atrial (LA) monophasic action potential (MAP) and local bipolar electrogram. Another Franz catheter was advanced to the right atrial (RA) free wall from the right femoral vein, to record the RA electrogram. Two pairs of silver wire hook electrodes were inserted into the RA appendage and right ventricle, respectively, for electrical stimulation. Pacing pulses, 2.0 ms in duration at twice the diastolic threshold, were delivered by a programmable cardiac stimulator. Upon completion of the experiments, the animals were sacrificed by electrical induction of ventricular fibrillation under deep general anesthesia.

Data Acquisition and Analysis

The signals from each intracardiac catheter were recorded on a MCS-5500 polygraph (Fukuda Densi Co Ltd, Tokyo, Japan) at a paper speed of 100 mm/s, using a filter setting of 0.05–500 Hz for MAP recordings and 50–500 Hz for bipolar electrograms. The MAP duration at 90% repolarization (MAP90) at a basic pacing length of 350 ms was measured as the index action potential duration. During the atrial pacing at 350 ms, atrioventricular conduction block was observed after administration of bepridil and during the application of VNS before administration of bepridil. However, we did not measure the pacing cycle length showing Wenckebach atrioventricular conduction in order to complete the experimental protocol within a reasonably short interval to best measure the pharmacological effects of bepridil. The interatrial conduction time (CT-ia) was measured between the stimulus artifact delivered in the RA appendage and the local LA activation at a pacing cycle length of 300 ms. The effective refractory period (ERP) of the right atrium was measured using standard techniques, with trains of 10 regular stimuli (S1) at a pacing cycle length of 300 ms, and extrastimuli (S2) delivered at a coupling interval (S1S2) shortened in steps of 2 ms. ERP-RA was defined as the longest S1S2 interval at which S2 failed to capture the atrium. AF was induced, using up to 2 extrastimuli as a basic pacing cycle length of 300 ms or rapid pacing up to 200 ms.

Measurements of HRV

Body surface electrocardiogram recordings were digitized at a sampling rate of 1,000 Hz by an ADX-002 analog-to-digital converter (ADTEC Inc, Tokyo, Japan) and stored in a personal computer. The cardiac cycle length was measured using

GM-View II R-wave detection software (Signalysis Ltd, Saitama, Japan). The MemCalc method^{16–18} was used to calculate the high-frequency (HF; 0.15–0.4 Hz) and low-frequency (LF; 0.04–0.15 Hz) components of HRV, using continuous 90-s samples of RR intervals, using MemCalc, Version 2.0, software (Suwa Trust Co, Tokyo, Japan). MemCalc combines a maximum entropy method for the spectral analysis and a non-linear least squares method for the fitting analysis, allowing the analysis of both LF and HF components over a minimum sampling interval of 30 s.^{16–18} In this study, the LF and HF components were expressed as their amplitude [LF-amp=(2 LF component)^{1/2} and HF-amp=(2 HF component)^{1/2}] and the LF/HF ratio was calculated.¹¹

Study Protocol and Data Collection

The experimental protocol was completed within 2 h after the surgical preparation and instrumentation of the animals. In this study, we focused on the inducibility of AF with or without application of VNS as a parameter of the antiarrhythmic effect of bepridil for AF.

Before Administration of Depridil Completion of the following protocols of A–C took approximately 60–90 min.

(A) First, MAP90 in both atria, CT-ia and ERP-RA were measured. Then, programmed electrical stimulation was applied to examine the inducibility of AF.

(B) Second, right VNS was gradually increased from 0.5 V in steps of 0.1 V, and the lowest voltage that induced AF for >30 s (VNS-min.V) was obtained.¹¹ Once AF was induced and sustained, VNS was discontinued within 45 s to avoid the development of electrical remodeling in the atrium.¹⁹ The VNS-min.V used in this study was 1.2±0.5 V (N=9). During the application of VNS-min.V, MAP90, CT-ia and ERP-RA were again measured. Demand back-up ventricular pacing at a cycle length of 1,000 ms was used to prevent profound bradycardia during all episodes of VNS.

(C) Third, RR intervals were recorded for analysis of HRV during spontaneous rhythm, in absence and during VNS-min.V. If the sinus rate fell below that of junctional escape during VNS-min.V, the output of VNS was slightly decreased until reemergence of sinus rhythm, to allow the sampling of RR intervals for analysis of HRV during VNS.

After Bepridil Administration Completion of the following protocols of A–B was within 30 min.

Bepridil, 4 mg/kg, was dissolved into sterile saline and infused intravenously over 5 min.

(A) First, 5 min after the administration of bepridil was completed, the RR intervals were sampled during sinus rhythm, before and during VNS, at an output identical to that delivered before drug administration.

(B) Second, MAP90, CT-ia and ERP-RA were measured, and the inducibility of AF was examined, before and during the application of VNS-min.V. If AF was induced, the mean F-F intervals during application of VNS-min.V before and after administration of bepridil were compared.

Statistical Analysis

Data are presented as means±SD. The electrophysiological measurements were compared using a paired t-test. The inducibility of AF was analyzed by Fisher's exact probability method. The variables of HRV were compared using Wilcoxon's signed-rank sum test. Differences in the hemodynamic and electrophysiological data among the 3 periods, ie, beginning, middle (approximately 15 min after starting the bepridil protocol) and completion of the experimental protocol of bepridil, were examined with repeated measures

Figure 1. (A) Induction of atrial fibrillation (AF) by programmed electrical stimulation. Electrical stimuli were delivered during vagal nerve stimulation (VNS-min.V). Before administration of bepridil (Upper), sustained AF was induced in all 9 experiments. After administration of bepridil, AF became non-inducible during the application of VNS-min.V in 6 of the 9 experiments (Lower). (B) Prolongation of F-F interval after administration of bepridil. Before administration of bepridil, the mean F-F interval of induced AF was 91 ms (Left). In this experiment, AF was again induced after the administration of bepridil. However, the mean F-F interval was lengthened to 158 ms (Right). Surface ECG (I), lead I of surface electrocardiogram; LA bi, left atrial bipolar electrogram; LA-MAP, left atrial monophasic action potential; RA bi, right atrial bipolar electrogram; RA-MAP, right atrial monophasic action potential; S_1 , basic pacing train; S_2 and S_3 , second and third premature stimuli; Surface ECG (aVF), lead aVF of surface electrocardiogram.

Table. Electrophysiological Measurement in the Atrium				
	Before bepridil (n=9)		After bepridil (n=9)	
	VNS off	VNS on	VNS off	VNS on
RR interval (ms)	523±104	735±190 [†]	725±97	950±166 [†]
	(+42±30%)		(+32±22%)	
ERP-RA (CL 300ms, ms)	133±23	74±13 [†]	180±19	150±21 [†]
	(-43±10%)		(-16±11%)	
RA-MAP90 (CL 350ms, ms)	162±22	110±9 [†]	184±33	166±39
	(-31±8%)		(-10±13%)	
LA-MAP90 (CL 350ms, ms)	169±19	133±24 [†]	190±15	179±25
	(-22±12%)		(-6±8%)	
CT-ia (CL 300ms, ms)	54±7	54±8	57±9	57±10
	(±0%)		(±0%)	
AF inducibility	0/9	9/9	0/9	3/9*
	(0%)		(0%)	

[†]P<0.05 vs control; *P<0.05 vs before drug administration with VNS.

VNS, vagal nerve stimulation; ERP-RA, effective refractory period of right atrium; CL, basic cycle length; RA-MAP90, the monophasic action potential duration at 90% repolarization (MAP90) in the right atrium; LA-MAP90, MAP90 in the left atrium; CT-ia, intra-atrial conduction time; AF, atrial fibrillation.

Figure 2. Measurement of atrial action potential duration. VNS-min.V shortened the monophasic action potential duration at 90% repolarization (MAP90) in both atria before bepridil (**Left panels**). After the administration of bepridil, MAP90 was prolonged, and its VNS-min.V-induced shortening was attenuated. Upstroke velocity of the MAP seemed to be decreased after the administration of bepridil, which may suggest the inhibitory effect of I_{Na} by bepridil. Abbreviations are as in **Figure 1**.

analysis of variance (ANOVA) with Bonferroni's correction. A P-value of <0.05 was considered statistically significant.

Results

Effects of Bepridil on Heart Rate, Blood Pressure and Electrocardiogram

Before the administration of bepridil, the mean sinus cycle length was 523±104 ms (N=9), and the mean systolic/diastolic blood pressure was 120±20/64±14 mmHg (N=9). Bepridil

prolonged the sinus cycle length to 725±97 ms (P<0.01, N=9) and decreased the systolic/diastolic blood pressure to 101±18/55±8 mmHg (P<0.05, N=9). The PQ interval lengthened from 110±12 to 131±7 ms (P<0.001, N=9), and the QT/QTc interval from 318±61/439±60 ms to 483±86/566±92 ms (P<0.01, N=9). No significant change was observed in the QRS duration (63±9 vs 65±10 ms, N=9).

Bepridil and Vagal Stimulation

Before the administration of bepridil, VNS-min.V prolonged

Figure 3. Heart rate variability (HRV) analysis. (**Upper**) HRV analysis before administration of bepridil. VNS-min.V increased high-frequency (HF)-amp and low-frequency (LF)-amp, although it had no significant effect on LF/HF. (**Lower**) Similar effects of VNS-min.V were observed after the administration of bepridil. See text for detailed explanations. Abbreviations are as in Figure 1.

the mean sinus cycle length from 523 ± 104 to 735 ± 190 ms ($P < 0.01$, $N=9$), and lowered only modestly the systolic/diastolic blood pressure from $120 \pm 20/64 \pm 14$ to $110 \pm 17/62 \pm 12$ mmHg (NS, $N=9$). After the administration of bepridil, VNS-min.V prolonged the mean sinus cycle length from 725 ± 97 to 950 ± 166 ms ($P < 0.01$, $N=9$), whereas the mean systolic/diastolic blood pressure remained nearly unchanged, from $101 \pm 18/55 \pm 8$ to $104 \pm 14/53 \pm 13$ mmHg (NS, $N=9$). The mean percentage increase in sinus cycle length induced by VNS-min.V was similar before ($+42 \pm 30\%$) vs after ($+32 \pm 22\%$) the administration of bepridil (NS, $N=9$).

Inducibility of AF

Before the administration of bepridil, programmed electrical stimulation alone failed to induce AF in all 9 animals. In contrast, sustained AF was induced by a single atrial extrastimulus during the application of VNS-min.V in all 9 experiments (Figure 1A). After the administration of bepridil, AF was non-inducible in all 9 animals before, and was induced in 3 of the 9 experiments during VNS-min.V, representing a 67% decrease in the inducibility of AF during VNS-min.V ($P < 0.01$). In the remaining 3 experiments, the mean F-F interval during VNS-min.V increased from 75 ± 14 ms before to 147 ± 13 ms after the administration of bepridil ($P < 0.05$, $N=3$; Figure 1B).

Electrophysiological Measurements in the Atria

At Baseline RA-MAP90 and LA-MAP90 measured 162 ± 22 ($N=9$) and 169 ± 19 ms ($N=9$), respectively, ERP-RA measured 133 ± 23 ms ($N=9$) and CT-ia 54 ± 7 ms ($N=9$) (Table).

VNS-min.V shortened MAP90 by a mean of $-31 \pm 8\%$ ($N=9$) in the RA and $-22 \pm 12\%$ ($N=9$) in the LA ($P < 0.01$, $N=9$; Figure 2), and ERP-RA by $-43 \pm 10\%$ ($P < 0.01$, $N=9$; Table), although it had no significant effect on CT-ia (Table).

After the Administration of Bepridil RA-MAP90, LA-MAP90 and ERP-RA duration increased to 185 ± 33 ms ($P < 0.05$, $N=9$), 190 ± 15 ms ($P < 0.05$, $N=9$) and 180 ± 19 ms ($P < 0.001$, $N=9$), respectively (Table). VNS-min.V shortened the RA-MAP90 by $-10 \pm 13\%$, LA-MAP90 by $-6 \pm 8\%$ ($N=9$) and ERP-RA by $-16 \pm 11\%$ ($N=9$). The degree of shortening in these measurements induced by VNS-min.V was significantly smaller ($P < 0.01$ for RA-MAP90 and LA-MAP90, $N=9$ and $P < 0.001$ for ERP-RA, $N=9$) than before the administration of bepridil. While bepridil prolonged CT-ia from 54 ± 7 to 57 ± 9 ms ($P=0.09$, $N=9$), no further significant change in CT-ia was observed after its administration during VNS-min.V (57 ± 9 ms vs 57 ± 10 ms, $N=9$).

HRV

Before the Administration of Bepridil VNS-min.V increased HF-amp from 1.2 ± 0.7 to 5.3 ± 4.0 ms ($P < 0.01$, $N=9$) and LF-amp from 1.1 ± 1.0 to 5.6 ± 3.9 ms ($P < 0.01$, $N=9$), while the LF/HF ratio increased non-significantly from 1.3 ± 2.0 to 1.5 ± 1.3 ($N=9$) (Figure 3).

After the Administration of Bepridil No significant effect was observed on LF-amp (from 1.1 ± 1.0 to 1.1 ± 0.9 ms, $N=9$), HF-amp (from 1.2 ± 0.7 to 1.7 ± 0.8 ms, $N=9$), or LF/HF ratio (from 1.3 ± 2.0 to 0.6 ± 0.7 , $N=9$) before VNS-min.V. During VNS-min.V HF-amp increased from 1.7 ± 0.8 to 5.4 ± 2.3 ms ($P < 0.01$, $N=9$), and LF-amp from 1.1 ± 0.9 to 5.7 ± 2.5 ms ($P < 0.01$, $N=9$).

0.01, $N=9$), leaving the LF/HF ratio nearly unchanged (0.6 ± 0.7 to 1.6 ± 1.5 , $N=9$; **Figure 3**). The mean magnitude of increase in HF-amp and LF-amp induced by VNS-min.V before vs after bepridil ($+393\%$ vs $+274\%$ and 810% vs $+704\%$, $N=9$) were not statically significant.

Discussion

The main findings of this study were: (1) bepridil suppressed the induction of AF during VNS-min.V in two-thirds of the animals, (2) bepridil limited the shortening of MAP90 during VNS-min.V in both atria and (3) the lengthening of the sinus cycle length and increase in HF-amp by VNS-min.V were not statically significant before and after the administration of bepridil.

Facilitation of AF Induction by VNS

Vagal activation is notoriously proarrhythmic in various types of AF.²⁰ This effect is attributed to a shortening of the activation wavelength without significant change in conduction velocity, allowing the coexistence of multiple activation wavefronts in the atria. Acetylcholine, released from the parasympathetic nerve terminals, enhances $I_{K_{ACh}}$, while limiting the pacemaker current by activating muscarinic M2 receptors in the atria. The increase in $I_{K_{ACh}}$ shortens atrial repolarization and action potential duration, and suppression of the pacemaker current slows the sinus rate. In this study, as in previous studies,^{11,21} the shortening of MAP90 in the atrium by VNS-min.V coincided with the inducibility of AF.

Pharmacological Therapy of AF

Inhibition of the sodium current (I_{Na}) and of the rapid (I_{Kr}) and slow (I_{Ks}) components of the delayed rectifier potassium current with class I or class III antiarrhythmic drugs are typical objectives of pharmacological suppression of AF. However, the efficacy of sodium channel blockers in preventing the initiation of AF is limited, while amiodarone, a multichannel blocker, is more effective in the suppression of AF.^{22,23}

Recent studies have reported a high efficacy of bepridil in the suppression of paroxysmal and persistent AF.^{24,25} As in the case of amiodarone, bepridil blocks multiple myocardial ionic channels. In this study, MAP90 lengthened in both atria after the administration of bepridil, probably by inhibiting I_{Kr} , I_{Ks} and the ultra rapid potassium current (I_{Kur}). In contrast, CT-ia was unchanged by the administration of bepridil, suggesting that the inhibitory effect of bepridil on I_{Na} in the atrium is weaker in the doses used in this study. However, the effects of I_{Na} blockade by antiarrhythmic drugs may be use-dependent. Therefore, when we measured CT-ia at a paced cycle length of 300 ms, no apparent inhibitory effect on I_{Na} was observed, whereas the F-F interval was lengthened by the administration of bepridil in the 3 animals in whom AF remained inducible after administration of the drug (**Figure 1B**).

Bepridil prolonged the spontaneous sinus cycle length and slightly decreased the systolic and diastolic blood pressures in absence of VNS-min.V. These effects were probably due to the inhibition of L and T type calcium currents (I_{Ca}). Conversely, the inhibition of $I_{K_{ACh}}$ in the sinus node by bepridil might have a counteractive effect in the prolongation of sinus cycle length to some degree.

Inhibition of $I_{K_{ACh}}$ and HRV

Anticholinergic antiarrhythmic drugs, such as disopyramide and cibenzoline, are effective in the treatment of vagally

mediated AF by preventing the shortening of the atrial action potential duration during VNS.²⁶ A similar observation was made in this study, although the electrophysiological mechanism appeared different from that associated with anticholinergic drugs. In our previous study, using the same model of AF, the VNS-induced prolongation of sinus cycle length and increase in HF-amp were counteracted by disopyramide, which effectively blocked the activation of muscarinic M2 receptors in the atrium.

In this study, the muscarinic M2 receptors appeared to be activated by VNS-min.V, as the prolongation of the sinus cycle length by VNS-min.V was statistically similar both before and after administration of bepridil. This impression was somewhat supported by the analysis of HRV, which revealed a similar degree of increase in HF-amp by VNS-min.V, before and after the administration of bepridil. In contrast, the shortening of MAP90 induced by VNS-min.V in both atria was markedly attenuated after the administration of bepridil. Therefore, we think that, as far as this study shows, it seems possible that inhibition of $I_{K_{ACh}}$ by bepridil played a part in this antifibrillatory effect. In a previous study using guinea-pigs, $I_{K_{ACh}}$ in atrial myocytes was inhibited by bepridil in a concentration-dependent manner.¹⁰ This inhibition of $I_{K_{ACh}}$ may be associated with the suppression of AF by bepridil. In addition, the statistically insignificant but non-trivial attenuation in the VNS-min.V induced prolongation of the sinus cycle length ($42 \pm 30\%$ vs $32 \pm 22\%$, $N=9$) and augmentation of the HF-amp ($393 \pm 349\%$ vs $274 \pm 256\%$, $N=9$) after bepridil administration could be associated with an inhibitory effect of $I_{K_{ACh}}$ in the sinus node by bepridil. Such specific electrophysiological effects to the sinus node can affect the results of HRV analysis. Therefore, HRV analysis in such a situation may limit the accuracy of any autonomic nerve activity measurement in regards to the atrial myocardium.^{27,28}

As with the other potential mechanisms, prolongation of the baseline MAP90 due to the inhibition of I_{Kr} , I_{Ks} and/or I_{Kur} could be associated with the cancellation effect of VNS-induced shortening of the MAP90 in the atrium. Furthermore, because bepridil has non-specific calcium channel blocking effects, calcium-mediated release of Ach from the nerve endings may be prevented through the inhibition of N-type calcium channel current to some degree.²⁹

Clinical Implications

The antifibrillatory effects conferred by bepridil in AF induced by VNS suggest that it might be the drug of choice for AF triggered by vagal nerve activation, particularly in patients suffering from disorders such as prostate hypertrophy or glaucoma. Because cholinergic actions of chemicals constrict smooth muscles, antiarrhythmic drugs that include the anti-cholinergic effect are usually unavailable for patients who suffer from prostate hypertrophy and/or glaucoma, etc. This is because anti-cholinergic action inhibits muscle constriction of the urinary bladder and can induce dysuria and constriction of the ciliary muscle and sphincter muscle of the pupil, which can be a cause of elevation of intraocular pressure. Dobrev et al found that $I_{K_{ACh}}$ is continuously increased in patients presenting with chronic AF.³⁰ Therefore, like several new antiarrhythmics, which inhibit I_{Kur} or atrial specific I_{Na} ,^{31,32} drugs with $I_{K_{ACh}}$ -blocking properties may play an important role in the management of AF.

Study Limitations

First, we used the right vagal nerve trunk to apply VNS. Because the right atrium is mainly innervated by the right vagal

nerve, unilateral nerve stimulation may have affected the results of our study. However, the absolute difference in MAP90 between RA and LA was similar before and during VNS-min.V (25 ± 18 ms during and 15 ± 13 ms before VNS-min.V, before bepridil, $P=0.26$, $N=9$ and 32 ± 31 ms during and 39 ± 35 ms before VNS-min.V, after bepridil, $P=0.31$, $N=9$; Table). Compared to some other studies,^{12,13} we used relatively higher Hz and lower output for the VNS. However, an obvious vagotonic effect was obtained by the stimulation and we have used this protocol in our previous experimental studies.^{14,15} Second, we did not monitor the serum and tissue concentration of bepridil in this study. Although we completed the experimental protocol after administration of bepridil within 30 min, the serum and tissue concentration of bepridil would certainly have altered during this period and, of course, this may have affected the results of the study. However, there was no statistical difference in the following parameters measured in the basic sinus rhythm among the beginning, middle (approximately 15 min after starting the study protocol of bepridil) and completion period of the experimental protocol of bepridil; basic cycle length (725 ± 97 , 710 ± 56 , 694 ± 65 ms, $P=0.899$ by ANOVA), blood pressure ($101 \pm 18/55 \pm 8$, $96 \pm 8/60 \pm 11$, $98 \pm 8/61 \pm 10$ mmHg, $P=0.805$ for systolic and $P=0.767$ for diastolic blood pressure by ANOVA), PQ interval (131 ± 7 , 128 ± 15 , 125 ± 15 ms, $P=0.673$ by ANOVA), width of QRS complex (65 ± 10 , 65 ± 5 , 67 ± 8 ms, $P=0.392$ by ANOVA), QT interval (483 ± 86 , 477 ± 57 , 460 ± 53 ms, $P=0.631$ by ANOVA), RA-MAP90 (218 ± 23 , 227 ± 35 , 220 ± 20 ms, $P=0.484$ by ANOVA) and LA-MAP90 (218 ± 23 , 223 ± 21 , 217 ± 21 ms, $P=0.755$ by ANOVA). Therefore, we think that the electrophysiological effects of bepridil were present during the experimental protocol. Third, it is usually difficult to obtain a stable MAP recording during the whole experimental period. Because we used Franz catheters to record the MAP from the atriums, it may be possible that activation of stretch activated channel, etc could have altered the configuration of MAP and modified the results of measurement.^{33,34} Perhaps, another recording method (eg, plunge electrode) would affect the analysis of the local repolarization in this experimental protocol to a lesser extent. Fourth, in this study, we focused on the inducibility of AF to estimate antiarrhythmic effects of bepridil. However, the duration and spontaneous terminability of AF are also important antiarrhythmic parameters in the treatment of AF. We performed programmed electrical stimulation from the RA appendage, although, in humans, the triggers of AF are known to frequently originate from the pulmonary vein, LA, or both.^{8,9} Furthermore, we used a relatively gentle protocol of AF induction, which might have amplified the antifibrillatory effects of bepridil. Fifth, the alteration of rate-dependent change in the MAP90 in both atria can be associated with the antifibrillatory effects of bepridil, but this parameter was not assessed in this study. Sixth, we analyzed HRV from 90-s samples of surface ECG. Although similar samples were used in previous studies,^{16–18} 90-s ECG segments might be too short to acquire reliable LF and HF components. Finally, although we administered low doses of sodium thiopental, anesthetic agents might influence the inducibility of AF and HRV analysis.

Conclusions

In this experimental study, prevention of VNS-induced shortening of the MAP90 in the atrium seems to play some role in the antiarrhythmic effect of bepridil on AF.

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