Bepridil Inhibits Sub-Acute Phase of Atrial Electrical Remodeling in Canine Rapid Atrial Stimulation Model

Daisuke Sato, MD; Shinichi Niwano, MD; Ryuta Imaki, MD; Yoshihiko Masaki, SB; Sae Sasaki, MD; Masaru Yuge, MD; Shoji Hirasawa, MD; Takeshi Sasaki, MD; Masahiko Moriguchi, MD; Hiroe Niwano, MD; Hirokuni Yoshimura, MD*; Tohru Izumi, MD

**Background** The effect of bepridil, a multichannel blocker, on atrial electrical remodeling was evaluated in a canine rapid atrial stimulation model.

**Methods and Results** In 10 beagle dogs, the right atrial appendage (RAA) was paced at 400 beats/min for 2 weeks. The atrial electrophysiological parameters, including effective refractory period (AERP), were evaluated at three atrial sites: RAA, the right atrium close to the inferior vena cava (IVC) and the left atrium (LA), during the time course of rapid pacing. Five of the dogs were given bepridil (10 mg·kg⁻¹·day⁻¹ po). In the control group, AERP was significantly shortened at all atrial sites and the AERP shortening (ΔAERP) was larger for the RAA and LA than at the IVC site (p<0.05). In the bepridil group, AERP was smaller than that of the controls at all atrial sites, and the AERP started to return slowly to the pre-pacing level in the second week, regardless of the continuation of rapid pacing.

**Conclusions** In a canine rapid atrial stimulation model, bepridil suppressed AERP shortening. Bepridil might have a reverse electrical remodeling effect, at least for AERP shortening, because it showed slow recovery of AERP in the subacute phase of rapid atrial pacing. (Circ J 2006; 70: 206–213)

**Key Words:** Atrial fibrillation; Bepridil; Electrical remodeling

Atrial electrical remodeling, which is characterized by shortening of the atrial effective refractory period (AERP) and a decrease in conduction velocity (CV), is considered to play an important role in the construction of arrhythmogenic substrates for atrial fibrillation (AF). We have documented the inhomogeneous process of electrical remodeling and the effects of verapamil and pilsicainide in a canine rapid atrial stimulation model. Although there were suppressive effects on electrical remodeling, AERP was still shorter than the baseline, indicating that electrical remodeling was not suppressed completely. Recently, the effect of amiodarone on electrical remodeling and the possibility of reverse electrical remodeling have been reported but the effect of class III antiarrhythmic agents on electrical remodeling is still controversial. Bepridil, a multichannel blocker with an IKₐ-channel-blocking effect, is attracting more clinical attention because of its effect on AF, especially in the subacute phase. In the present study, the effect of bepridil on electrical remodeling was evaluated in a canine rapid atrial stimulation model.

**Evaluation of Electrophysiological Properties**

To achieve a stable condition, each dog was allowed to recover for 7 days after the initial surgical procedure without any pacing. Then, rapid atrial pacing (400 beats/min) was initiated and continued for 2 weeks (rapid pacing phase). This pacing was performed at an output of 4-fold the diastolic threshold and a pulse width of 2 ms. After this continuous rapid pacing, the pacing was ceased, and each dog was allowed to recover for 1 week (recovery phase). On days 0, 3, 7, 10 and 14 during the rapid pacing phase, the rapid pacing was stopped temporarily to evaluate the atrial surface was exposed via a right thoracotomy under pentobarbital anesthesia (30 mg/kg bodyweight, iv) and mechanical ventilation (Model SN-480-5, Shinano Manufacturing, Tokyo, Japan) with oxygen (2 L/min). Three pairs of stainless steel wire electrodes were sutured onto the epicardial surface of the right atrial appendage (RAA), the right atrium close to the inferior vena cava (IVC) and the center of the left atrial free wall (LA) (Fig 1A). The other ends of the wire electrodes were tunneled subcutaneously and exposed at the back of the neck. These electrodes were used for electrophysiological measurements in the later studies. For continuous atrial rapid pacing, a screw-in pacing electrode (CapSureFix 5568, Medtronic Inc, Minneapolis, MN, USA) was fixed on the endocardial surface of the RAA through the right external jugular vein. The distal end of this pacing electrode was connected to a rapid pulse generator (customized Thera SR, Medtronic), which was implanted subcutaneously in a right cervical lesion! All studies were performed in accordance with the guidelines specified by the Institutional Animal Care and Use Committee of the Kitasato University School of Medicine.
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Circulation Journal  Vol.70, February 2006

electrophysiological properties at the 3 sites (ie, RAA, IVC and LA). On days 1, 3 and 7 during the recovery phase (ie, days 15, 17 and 21 from the start of rapid pacing), the electrophysiological properties were similarly evaluated. All electrograms were recorded through a polygraph system (Bioelectric AMPL, NEC, Tokyo, Japan). The analog signals were converted to digital signals and stored on a computer hard-disk (Power Lab, ADInstrument, USA) and subsequently used for analysis. At each evaluation point, all measurements were performed after the pharmacological blocking of autonomic nervous system by infusing atropine 0.04 mg/kg and propranolol 0.2 mg/kg.

Atrial Diastolic Threshold  At each evaluation of the electrophysiological parameters, the atrial diastolic threshold was measured at the 3 pacing sites by delivering 300 ms cycle length pacing with a pulse width of 2 ms.

AERP  AERP was measured at the 3 pacing sites with basic drive cycle lengths (BCL) of 300, 200 and 150 ms at each evaluation time as described earlier. The pacing energy was 2-fold the diastolic threshold at each pacing site at each time of the evaluation. The coupling interval of the premature stimulus was shortened by 2 ms steps. The longest coupling interval of the premature beat that failed to capture the atrium was determined as the local AERP.

Dispersion of the AERP (AERPd)  In the present study AERPd was evaluated as an index of the inhomogeneity of

Fig 1. Schematic of the positions of the atrial electrodes (Panel A) and (Panel B) experimental protocol (see text for details). Position of the 3 epicardial pacing wires (●) and of the endocardial pacing lead (○). LA, left atrium; PV, pulmonary vein; SVC, superior vena cava; IVC, inferior vena cava; RAA, right atrial appendage.

Fig 2. Atrial effective refractory period (AERP) in the control state. The AERPs at each pacing site on day 0 (ie, 1 week after surgery and before the start of rapid pacing) showed no significant difference between the control and bepridil groups. For all 3 basic cycle lengths, the AERP was shorter at the left atrial site than the other sites. See text for details.

*p<0.05 LA vs right atrium, IVC; BCL, basic cycle length; RAA, right atrial appendage; IVC, right atrium close to the inferior vena cava; LA, left atrium; RA, right atrium.
atrial refractoriness during the progression and/or recovery of atrial electrical remodeling. AERPd was calculated as the difference between the longest and shortest AERPs of the 3 pacing sites, and was evaluated with each BCL at each evaluation time during the whole study protocol.

**CV in the Atrium** Conduction time between the RAA and LA sites was measured during RAA pacing at cycle lengths of 300, 200 and 150 ms. CV was calculated as a reciprocal of conduction time.

**Inducibility of AF** To evaluate the inducibility of AF, its incidence and duration were evaluated with atrial burst pacing for 3 s at the minimal pacing cycle length that achieved a 1:1 atrial capture at each pacing site. This pacing was delivered at 4-fold the diastolic threshold with a pulse width of 2 ms. In this study AF was defined as spontaneous irregular atrial rhythm lasting longer than 1 s. Atrial burst pacing for AF induction was delivered 5 times at each pacing site at each evaluation point during the whole protocol.

**Ventricular and Atrial Heart Rate** To roughly evaluate the hemodynamics during rapid pacing, ventricular heart rate (V-HR) was measured before each evaluation of the electrophysiological properties (ie, before stopping the rapid pacing and pharmacological autonomic blocking). The mean V-HR was measured from 30 s recording during rapid atrial pacing. At the same time, atrial heart rate (A-HR) at each recording site was evaluated to confirm the capture of each atrial site by rapid pacing.
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Administration of Bepridil

Five dogs were given oral bepridil (10 mg·kg⁻¹·day⁻¹), starting 7 days before the rapid atrial pacing protocol began. This starting point of the medication was decided in accordance with data (unpublished) from an evaluation of the plasma concentration of bepridil in 6 healthy males. The same evaluation protocol was used for the administration of bepridil in the present study (Fig 1B).

Statistical Analysis

Values are expressed as means±SD. Basic comparative statistics were analyzed with a 1-way ANOVA test or paired t-test. A p-value <0.05 was considered significant.

Results

Atrial Diastolic Threshold

The atrial diastolic threshold was measured at 3 pacing sites during each electrophysiologic evaluation. In the control group at day 0, the values were 0.9±0.2 V, 0.9±0.1 V and 0.9±0.2 V at the RAA, IVC and LA sites, respectively. In the bepridil group, the respective values were 1.0±0.5 V,
1.2±0.8 V and 0.9±0.6 V. There was no significant difference between the 2 groups and the results did not show any significant changes throughout the study protocol.

**Plasma Concentration of Bepridil in the Bepridil Group**

The plasma concentration of bepridil was 357±260 ng/ml on day 0, 504±368 ng/ml on day 7 and 541±449 ng/ml on day 14, which shows that the concentration for maintenance therapy was achieved from the beginning of the rapid pacing.

**AERP**

Fig 2 shows the AERPs at the 3 sites on day 0 (ie, before the start of the rapid right atrial pacing protocol). Although the LA site showed significantly shorter AERP than the other atrial sites at all BCLs (p<0.05), as previously described, there was no significant difference between the control and bepridil groups.

Fig 3 shows the changes in AERPs at each site over the time course of the pacing protocol. The vertical axis indicates ΔAERP, which was calculated as the difference between AERPs at each evaluation point and the pre-rapid pacing state. In the control group, AERP shortening was relatively quick in the first 3 days and then continued to shorten until day 14. During the recovery phase, after the cessation of continuous rapid atrial pacing, AERP showed quick recovery within the first day and returned to the pre-rapid pacing state by day 15. ΔAERP was larger with longer basic cycle lengths. In the control group, AERP shortening was larger at the RAA site in comparison with the other 2 sites with the 300 ms BCL. In contrast, in the bepridil group, AERP was also shortened in the initial 7 days but the ΔAERP was relatively smaller than the control, especially at the RAA site with longer BCL, although

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**Incidence of induction of atrial fibrillation (AF)** (Left panels) and duration of induced AF (right panels) at the 3 atrial pacing sites at each evaluation point during the time course of the study. The incidence of AF induction became higher in accordance with the time course of rapid pacing in both groups, and the incidence of AF induction at the LA and RA sites was higher than that at the IVC site. In the bepridil group, the incidence of AF induction was suppressed at the LA and RA sites on day 14. The duration of induced AF did not show significant difference between the 2 groups, although it tended to be shorter in the bepridil group. See text for details. *p<0.05 Control vs Bepridil; +p<0.05 vs RA, IVC, RAA, right atrial appendage; IVC, right atrium close to the inferior vena cava; LA, left atrium; RA, right atrium.
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The difference was not significant. More interestingly, AERP started to recover slowly to the pre-pacing level in the second week (i.e., in the subacute phase), and then the ∆AERP became significantly smaller than the control at all atrial sites with all BCL.

**AERPd**

Fig 4 shows the changes in AERPd among the 3 sites with the 3 BCL. In the control group, the AERPd increased quickly in the first 3 days and was then maintained at that level until day 14. However, in the recovery phase, the AERPd decreased quickly and became temporarily smaller than in the pre-pacing state. In contrast, in the bepridil group, although each atrial site showed a relatively lower A-HR, there was no significant difference between the 2 groups. See text for details. No significant difference. RAA, right atrial appendage; IVC, right atrium close the inferior vena cava; LA, left atrium.

**Atrial CV**

Fig 5 shows the changes of CV in the atrium over the time course of the pacing protocol. In both groups, CV shortened gradually with rapid pacing and recovered after the cessation of the pacing. There was no significant difference between the 2 groups, although CV in the bepridil group tended to be smaller than the control at several time points.

**Inducibility of AF**

Fig 6 shows the incidence and duration of induced AF at the 3 sites at each evaluation point. In both groups, the incidence of AF induction became higher in accordance with the control value on day 14.

**Ventricular Heart Rate (V-HR)**

Fig 7. Changes in ventricular heart rate (V-HR) during the study protocol before pharmacological autonomic blocking. In both groups, a relatively high V-HR was maintained during rapid pacing and it returned to a lower level when sinus rhythm recovered. There was no significant difference between the 2 groups. See text for details. No significant difference.
the time course of rapid pacing and the incidence of AF induction at the LA and RA sites was higher than that at the IVC site. In the bepridil group, the incidence of AF induction was suppressed at the LA and RA sites at day 14. In contrast, the duration of induced AF did not show a significant difference between the 2 groups, although the duration of AF in bepridil group tended to be shorter than in the control. All induced AF was spontaneously terminated and persistent AF was not induced in the present study.

**V-HR and A-HR**

Fig 7 shows the change in V-HR during the study protocol before pharmacological autonomic blocking. In both groups, a relatively high V-HR was maintained during rapid pacing because of rapid atrial activation and relatively frequent conduction through the atrioventricular node. In the recovery phase, the V-HR returned to a lower level because sinus rhythm recovered. There was no significant difference between the 2 groups during the rapid pacing and the recovery phase. Fig 8 shows the change in the rate of atrial electrograms at each recording site during rapid pacing. In the control group, all atrial sites were completely captured by rapid pacing of 400 beats/min. In the bepridil group, although each atrial site showed a relatively lower A-HR than 400 beats/min, probably because of some capture and/or conduction failure, there was no significant difference between the 2 groups.

**Discussion**

**Effect of Bepridil on Preventing the Promotion of the Electrical Remodeling**

It has been reported that in a canine rapid atrial stimulation model atrial electrical remodeling was promoted by frequent atrial activation as well as atrial wall stretch, as a result of changes in hemodynamics. Although the correlation of various ion channels and/or intercellular junctions, such as I_{Kur}, I_{Ca-L}, I_{Ks}, I_{Na}, Cx42 etc, to electrical remodeling has been reported, the initial key to these changes is intracellular Ca^{2+} overload, especially in the earlier phase. In our model and/or similar animal models of rapid atrial stimulation, the suppressive effects of L- or T-type Ca^{2+} channel blockers have been reported and the mechanisms of these effects are explained by a reduction in the intracellular Ca^{2+} concentration. We have also reported the suppressive effect of pilocarpine, a pure sodium channel blocker, and its mechanism can also be explained as a reduction of intracellular Ca^{2+} caused by enhanced Na^{+}-Ca^{2+} exchange. Recently, the effect of amiodarone on atrial electrical remodeling was reported considering the action of class III antiarrhythmic drugs, amiodarone would be expected to worsen the Ca^{2+} overload by prolonging the action potential duration (APD), but surprisingly, it suppresses atrial electrical remodeling, especially in the subacute phase. This is partly explained by the decrease in the vagal stimulation effect caused by inhibition of I_{K-ACh}. Additionally, the effect of amiodarone on I_{Kur}, the main determinant of APD in the atrial myocardium, is relatively weak, so that the APD prolongation in the atrium would be expected to be small.

Bepridil, which is a multichannel blocker of I_{Kur}, I_{Na}, I_{Ca-L}, and I_{Ca-T} among others, is gaining attention because of its remarkable ability to terminate drug-refractory, persistent AF. From the point of view of atrial electrical remodeling, bepridil would be expected to suppress APD shortening directly by blocking I_{Kur}, and indirectly by reducing Ca^{2+} overload via I_{Ca-L} and I_{Ca-T} blocking, but it may worsen the decrease in CV by its action of Na^{+} channel blocking. However, APD prolongation as a result of I_{Kur} blocking may result in an increase of Ca^{2+} handling, so the long-term effect of bepridil on atrial electrical remodeling is controversial. In the present study, we evaluated the effect of bepridil on the changes in AERP and CV in a canine rapid atrial stimulation model, and the result was that bepridil suppressed AERP shortening in the first week, but more interestingly, AERP started to slowly prolong in the second week. CV was not affected by the administration of bepridil. This is the first documentation of the suppressive effect of bepridil on atrial electrical remodeling, at least for AERP shortening. AERPd, which is considered to be a result of inhomogeneity of AERP shortening, was also suppressed by bepridil administration.

In accordance with the classical definition of the atrial electrical remodeling, changes in the atrial electrophysiological properties are represented by AERP shortening and a decrease in CV. Both changes result in a decrease in wavelength and it becomes easier for a reentrant circuit to form. In the present study, bepridil suppressed AERP shortening and tended to prolong the AERP but did not affect CV, so its suppressive effect on atrial electrical remodeling might be considered partial.

**Effect of Bepridil in the Subacute Phase of Rapid Atrial Pacing**

The most impressive finding in the present study was the prolongation or recovery of the shortened AERP during the subacute phase of rapid pacing. This has not been previously observed with other antiarrhythmic agents. For example, in our previous reports verapamil or pilocarpine suppressed AERP shortening in the same model, but both drugs could only delay the progression of atrial electrical remodeling and could not return AERP to the pre-pacing state. This may explain the observation of a “reverse remodeling” effect of bepridil, at least for the AERP shortening, which we suspect occurs in clinical cases of drug-refractory, persistent AF.

Another possibility is that there was an insufficient concentration of bepridil at the beginning of the rapid pacing. In that scenario, AERP prolongation would be explained by a gradual increase in the bepridil concentration. In a basic experiment, 7 days should be a suitable period to obtain a stable plasma concentration of bepridil administered orally. In the present study, we confirmed that the plasma concentration of bepridil on day 0 (ie, the starting point for rapid pacing) had reached the therapeutic level and achieved the plateau on day 7 at least. Additionally, AERP on day 0 in the bepridil group tended to be longer than in the control, although this difference did not reach significance. Therefore, we considered the acute or direct effect of bepridil on the atrial myocardium appeared from the beginning of rapid pacing. However, because we could not check the tissue concentration of bepridil in this study, a gradual increase in the tissue concentration of bepridil is one possible explanation for the gradual prolongation of AERP in the subacute phase in this study.

The mechanism of “reverse remodeling” or re-prolongation of AERP in the subacute phase in this model is unclear. The intrinsic effects of bepridil on I_{Kur}, I_{Ks}, I_{Na} or I_{Ca-L} are not a direct explanation because they are considered to occur in the acute phase, depending on the tissue...
concentration. In the case of amiodarone or mibebradil administration, effects on T-type Ca²⁺ channels have been emphasized but this is considered a mechanism for the prevention, not the reversal, of electrical remodeling. We speculate that this effect is explained by upregulation of the ion channels, which are responsible for atrial electrical remodeling, but the expression level of each ion channel must be evaluated to validate this hypothesis.

**Study Limitations**

First, because His-bundle ablation was not performed, the influence of hemodynamic changes during rapid atrial pacing was not excluded. However, this model mimics actual clinical atrial tachyarrhythmia. Second, the expression of the ion channels or transporters was not evaluated. Finally, the role of the changes in the electrophysiological parameters (ie, AERP shortening and AERPd) in stimulating the appearance of AF was unclear. These parameters should be evaluated separately in future studies.

**Conclusions**

The effect of bepridil on electrical remodeling was evaluated in a canine rapid atrial stimulation model. Bepridil suppressed AERP shortening at all atrial sites and AERPd decreased, but CV was unaffected. AERPs showed slow recovery to the pre-pacing level in the subacute phase of stimulation of the ion channels or transporters was not evaluated. It is speculated that this effect is explained by upregulation of the ion channels or transporters.

**Acknowledgments**

This study was supported by a grant for scientific research from the Ministry of Education Science and Culture of Japan (No.16590713), and a grant to the Research Committee for Epidemiology and Etiology of Idiopathic Cardiomyopathy from the Ministry of Health, Labor and Welfare of Japan.

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