Inhomogenic Effect of Bepridil on Atrial Electrical Remodeling in a Canine Rapid Atrial Stimulation Model

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Background The antiarrhythmic or reverse remodeling effects of bepridil, a multi-ion channel blocker, have been recently reported, but inhomogeneity of the electrical remodeling and effects of bepridil have been observed in previous reports. In this study, the effect of long-term administration of bepridil on atrial electrical remodeling was evaluated in a comparison of the right and left atrium (RA and LA) in a canine rapid atrial stimulation model.

Methods and Results In 10 beagle dogs, rapid atrial pacing (400 beats/min) was delivered for 6 weeks and the atrial effective refractory period (AERP), conduction velocity (CV) and inducibility of atrial fibrillation (AF) were evaluated every week. In 5 of the pacing dogs, bepridil (10 mg·kg⁻¹·day⁻¹) was administered orally, starting 2 weeks after the initiation of the rapid pacing. At the end of the protocol, the hemodynamic parameters and extent of tissue fibrosis were evaluated and the mRNA of SCN5A, Kv4.3, the L-type Ca²⁺ channel (LCC) and connexin (Cx) 40, 43, and 45 in both atria were examined by quantitative real-time reverse transcriptase-polymerase chain reaction. In the pacing control group, AERP shortening, decreased CV, increased AF inducibility and downregulation of the expression of SCN5A and LCC were observed. In the bepridil group, the AERP exhibited a relatively quick recovery after bepridil was started in the first week and continued to recover gradually until the end of the protocol, but that recovery was smaller in the LA than in the RA. The CV was not affected by bepridil administration. AF inducibility was well suppressed in the RA in the bepridil group, but the induction of short-duration AF could not be suppressed in the LA. The mRNA downregulation of the LCC and SCN5A was negated by bepridil administration in the RA; but not in the LA; however, the data showed similar tendencies. There were no significant differences in the hemodynamic parameters or tissue fibrosis and the mRNA expression of Kv4.3, Cx40, 43, and 45 between the pacing control and bepridil groups.

Conclusion Bepridil exhibited an anti-electrical remodeling effect in this study as previously reported, but the effect was inhomogeneous between the RA and LA, with the LA appearing to be more resistant to the effect of bepridil. (Circ J 2008; 72: 318 – 326)

Key Words: Atrial fibrillation; Bepridil; Electrical remodeling

Although the importance of automatic triggers originating from the pulmonary veins has been emphasized in clinical cases of atrial fibrillation (AF), the electrophysiological properties of the atria themselves are considered to play an important role in maintaining the multiple random reentrant circuits of AF. Because atrial remodeling, including changes in the electrophysiological properties or atrial structure, may exaggerate the occurrence of AF, controlling this process should be one of the important issues in the management of clinical AF. There have been several reports focusing on preventing the progression of atrial electrical remodeling by various drugs, such as antiarrhythmic agents or angiotensin-receptor blockers. Some have been reported as effective for the partial suppression of electrical remodeling, but a more important issue is the recovery of the electrophysiological properties after the promotion of electrical remodeling because most clinical therapies would be instituted after the appearance of AF.

Bepridil hydrochloride is a multi-ion channel blocker that was originally developed as an anti-anginal drug. Several recent reports have documented the usefulness of this drug as an antiarrhythmic agent, especially for long-lasting drug-refractory persistent AF. Because there have been cases in which bepridil interrupted persistent AF after being administered for 1–2 months, a type of “reverse remodeling effect” has been speculated regarding the action of bepridil. In our previous experimental studies, we demonstrated that bepridil suppressed the shortening of the atrial effective refractory period (AERP) in the first week and continued to prolong it in the second week of a 2-week rapid pacing model, but the study design was not appropriate for documenting such a “reverse remodeling effect”. Most recently, Nishida et al reported that in their study in which they administered bepridil for 3 weeks after the start of rapid atrial pacing, it “reversed” electrical remodeling by suppressing the downregulation of expression of the L-type Ca²⁺ channel.
(LCC), but they did not document any difference between the right and left atrium (RA and LA). Because we have demonstrated an inhomogeneity of electrical remodeling in the RA and LA, at least in our canine rapid atrial stimulation model without atrioventricular block, the present study we focused on the effect of bepridil on atrial electrical remodeling and the difference between the RA and LA in using a new protocol in which we started administering the drug in the later phase of the pacing protocol.

Methods

Initial Surgery

Thirteen adult female beagle dogs (12.5±1.1 kg) were anesthetized with pentobarbital (30 mg/kg IV). Mechanical ventilation was maintained via an endotracheal tube using a mechanical ventilator (Model SN-480-5, Shinano Manufacturing, Tokyo, Japan) with 100% oxygen. Two pairs of stainless steel wire electrodes were sutured against the epicardial surface of the RA free wall within the pectinate muscle area and left atrial appendage. The other ends of the wire electrodes were tunneled subcutaneously and exposed at the back of the neck. For continuous rapid atrial pacing, a unipolar screw-in pacing lead (CapSureFix 5568, Medtronic Inc, Minneapolis, MN, USA) was inserted through the right external jugular vein, and the distal end of the lead was screwed into the endocardial side of the right atrial appendage (RAA). The proximal end of the pacing lead was connected to a rapid pulse generator (Soletra®, Medtronic Inc), which was implanted into a subcutaneous pocket in the neck. Atrioventricular block was not produced in this study in order to mimic the hemodynamic situation of clinical cases of AF. All studies were performed in accordance with the guidelines specified by the Animal Experimentation and Ethics Committee of the Kitasato University School of Medicine.

Evaluation of the Electrophysiological Properties

To obtain stable baseline conditions, each dog was allowed to recover after the initial surgical procedure for at least 1 week without pacing. In 10 dogs, rapid atrial pacing (400 beats/min) was initiated after this recovery period and continued for 6 weeks. Continuous rapid pacing was not performed in the remaining 3 dogs, which comprised the non-pacing control group, for the evaluation of the histology and mRNA expression. In the 10 dogs with continuous rapid pacing, atrial pacing was performed at an output of 4-fold the diastolic threshold and with a pulse width of 2 ms. The AERP, conduction velocity (CV), inducibility of AF by burst pacing, and duration of induced AF were evaluated every week, as were the ventricular response rates during rapid atrial pacing. All electrograms were recorded using a polygraph system (Bioelectric AMPL, NEC, Tokyo, Japan). Analog signals were converted to digital signals, stored on a computer hard-disk (Power Lab, ADInstrument, CO, USA) and subsequently subjected to analysis. During these studies, rapid pacing was stopped temporarily, and all measurements were performed after pharmacological block of the autonomic nervous system had been produced by infusing atropine 0.04 mg/kg and propranolol 0.2 mg/kg.

AERP At each evaluation time point, the AERP was measured at the 2 atrial sites where the electrodes were sutured, with basic drive cycle lengths (BCL) of 300, 200, and 150 ms. The pacing energy output was set at twice the diastolic threshold during each evaluation at each pacing site. 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.

% CV The conduction time between the RA and LA was measured during the pacing at cycle lengths of 300, 200, and 150 ms. The CV was calculated as the reciprocal of this conduction time, and the values were expressed in percentages as the % CV by dividing each piece of data by the data for day 0 to exclude the influence of the difference in the actual distance of the electrodes in each dog.

AF Inducibility and the Longest Duration of AF To evaluate AF inducibility, the incidence of AF induction was evaluated with atrial burst pacing for 3 s at the minimal pacing cycle length that achieved 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. When AF was induced, its duration was measured. We defined AF as a spontaneous irregular atrial rhythm lasting longer than 1 s, but the duration of the induced AF was subclassified as long (>10 s), medium (5–10 s), or short (1–5 s). The atrial burst pacing for AF induction was delivered 5 times at each pacing site at each evaluation time point during the entire protocol.

Administration of Bepridil

Bepridil (10 mg·kg⁻¹·day⁻¹) was orally administered to 5 of the 10 dogs undergoing continuous rapid atrial pacing starting 2 weeks after the initiation of rapid pacing, and they were assigned as the bepridil group. This protocol was designed to evaluate the effect of bepridil on atrial electrical remodeling as a “downstream therapy” for the AF substrate promoted in the initial 2 weeks before bepridil administration. All other protocols were similarly performed in the other 5 dogs with continuous rapid pacing and they were assigned as the pacing control group.

Hemodynamic Parameters

On day 42, the end of the protocol, the following hemodynamic parameters were evaluated by catheterization: systemic blood pressure (BP), pulmonary arterial pressure (PAP), pulmonary arterial wedge pressure (PAWP), central venous pressure (CVP), and cardiac output.

Histology

At the end of the protocol, all dogs, including the non-pacing control dogs, were killed and small portions of the RA and LA free walls were excised from the area close to the electrodes and fixed for histological analysis. Tissue fibrosis was evaluated by Azan staining, and fibrosis was expressed as the mean – %area on digitized images.

Quantitative Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Small portions of both atria were sampled from the area close to the electrodes at the end of the protocol. The total RNA was prepared from the RA and LA free walls using a total RNA isolation kit (SV Total RNA Isolation System, Promega, Madison, WI, USA). Complementary deoxyribonucleic acid (cDNA) was synthesized from 3 μg of the total RNA with reverse transcriptase (Invitrogen, San Diego, CA, USA) in a final volume of 20 μl. The mRNA levels of the ion-channel-related molecules (ie, LCC, Kv4.3, and SCN5A) and gap-junction-related molecules (ie, connexin (Cx) 40, 43, and 45) were evaluated by quantitative real-time RT-PCR. These factors were chosen
Table 1 PCR Primers Used for Amplification of Ion-Channel and Cx-Related Genes

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<th>Antisense</th>
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<td>5'-CATCACCAGCTCAGTAAGAG</td>
</tr>
<tr>
<td>SCN5A</td>
<td>5'-ATTAAAAGCTGGGATTGGA</td>
<td>5'-GAAGATCACCCGGAATGGA</td>
</tr>
</tbody>
</table>

PCR, polymerase chain reaction; LCC, L-type Ca2+ channel; Cx, connexin. *Control.

Fig 1. Changes in the atrial effective refractory period (AERP) over the time course in the groups with and without the bepridil administration starting at 2 weeks after the initiation of rapid pacing. In each panel, the vertical axis indicates the ∆AERP, which was calculated as the difference between the AERPs at each evaluation point and that in the pre-rapid pacing state, and the horizontal axis shows the time after starting rapid atrial pacing. The upper panels indicate the results in the right atrium (RA) and the lower panels those in the left atrium (LA). In the RA site, the AERP in the bepridil group exhibited a relatively quick prolongation in the first week after drug administration (p<0.05: day 14 vs day 21) and kept showing a gradual recovery until the end of the protocol (p<0.05: day 14 vs day 28, 35, 42, respectively). Comparing both groups, the differences became significant after day 21 of administration of bepridil (p<0.05: control vs bepridil group at day 21, 28, 35, and 42, respectively). In contrast, in the LA site, although the AERP in the bepridil group exhibited a similar tendency as in the RA site, the prolongation of the AERP at several time points was insignificant (vs the data for day 14 and vs the data at the same point in the pacing control group). See text for the details. BCL, basic cycle length.

Results

Time Course of Changes in the AERP

Fig 1 shows the AERP changes at the RA and LA sites during the time course of rapid atrial pacing. In the pacing control group, the AERP rapidly shortened during the first week (eg, day 0 vs day 7=0±0 vs –15.2±5.8 ms at the RA site with a 300 ms BCL, p<0.05), and continued to gradually shorten at both the LA and RA sites until the end of the protocol. The degree of AERP shortening was larger at the LA site than at the RA site, as previously reported. In the RA site, the AERP shortening was the same as in the pacing control group at the same point (p<0.05 vs day 21). In the pacing control group, the AERP rapidly shortened during the first week (eg, day 0 vs day 7=0±0 vs –15.2±5.8 ms at the RA site with a 300 ms BCL, p<0.05), and continued to gradually shorten at both the LA and RA sites until the end of the protocol. The degree of AERP shortening was larger at the LA site than at the RA site, as previously reported. In the bepridil group, the AERP shortened in the LA site with a similar tendency as in the RA site, the prolongation of the AERP at several time points was insignificant (vs the data for day 14 and vs the data at the same point in the pacing control group). See text for the details. BCL, basic cycle length. *p<0.05 vs the pacing control group at the same point; †p<0.05 vs day 14 in the bepridil group; ††p<0.01 vs day 0 in the pacing control group; †††p<0.001 vs day 0 in the pacing control group; ††††p<0.0001 vs day 0 in the pacing control group; †p<0.05 vs day 0 in the bepridil group; ††p<0.01 vs day 0 in the bepridil group.

Statistical Analysis

The values are expressed as mean±SE. The basic comparative statistics were analyzed with a 1-way ANOVA test or paired t-test. A p-value <0.05 was considered significant.
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Fig 2. Changes in percentage expression of conduction velocity (%CV) over the time course in the groups with and without the bepridil administration at 3 basic cycle lengths (BCLs). The %CV was significantly slower over the time course than that measured on day 0 in both groups. Furthermore, there was no significant difference between the 2 groups for any BCL. See text for the details. †p<0.05 vs day 0 in each group; ††p<0.01 vs day 0 in each group.

Fig 5. Changes in the inducibility of atrial fibrillation (AF) over the time course in the right atrium (RA, Left panel) and left atrium (LA, Right panel). For each evaluation, the left bar indicates the data for the pacing control group and the right bar shows the data for the bepridil group. The solid, gray and white bars indicate AF of long (>10 s), medium (5–10 s) and short (1–5 s) duration, respectively, and the total height of the bar indicates the sum of the incidence of induced AF (>1 s). In the RA, AF inducibility increased over the time course in the pacing control group and was significantly suppressed by bepridil administration. In the LA, AF inducibility also increased over the time course, but the inducibility of short-duration AF tended to be higher than in the RA from day 0. In the bepridil group, the inducibility of long-duration AF gradually decreased after bepridil administration, but the inducibility of shorter duration AF duration was not obviously suppressed. See text for the details. *p<0.05 vs pacing control group at the same point; †p<0.05 vs day 0 in the pacing control group; †p<0.05 vs day 0 in the bepridil group; †p<0.05 vs day 14 in the bepridil group with long-lasting AF (>10 s).

rapid prolongation in the first week (day 14 vs day 21=−29.6±9.9 vs −7.2±7.9 ms with a 300-ms BCL, −24.8±9.1 vs −4.4±5.7 ms with a 200-ms BCL, and −18.8±7.3 vs −5.4±6.1 ms with a 150-ms BCL, p<0.05) and (ii) a continued gradual prolongation over the later weeks (day 14 vs day 28, 35, and 42 at all BCLs, p<0.05). In the LA site, the tendencies of the changes in the AERP were the same, but the degree of change in the AERP was not obvious in comparison to that at the RA site. For example, the AERP was significantly prolonged at day 21 in comparison with day
Fig 4. Mean duration of induced atrial fibrillation (AF) in the right atrium (RA) and left atrium (LA) sites. The duration of the induced AF gradually increased over the time course in both atria in the pacing control group. In the bepridil group, the duration of the induced AF shortened from the first week after starting drug administration. These differences were significant in both atria. See text for the details. *p<0.05 vs pacing control group at the same point; †p<0.05 vs day 0 in the pacing control group; ††p<0.01 vs day 0 in the pacing control group; †††p<0.001 vs day 0 in the pacing control group; ††††p<0.0001 vs day 0 in the pacing control group.

AF Inducibility and the Duration of AF

Fig 3 shows the changes in AF inducibility in the RA and LA over the time course of the pacing protocol. When AF was induced from a site in the RA, inducibility increased over the time course in the pacing control group [8.0±1.7% (day 0) vs 56.0±8.6% (day 7), 72.0±6.0% (day 14), 74.0±5.2% (day 21), 80.0±5.6% (day 28), 88.0±3.5% (day 35), and 92.0±2.1% (day 42), p<0.05] and was significantly suppressed by bepridil administration [72.0±6.6% (day 14) vs 24.0±3.3% (day 21), 24.0±1.8% (day 28), 24.0±7.1% (day 35), and 16.0±5.2% (day 42), p<0.05]. Therefore, when comparing both groups, the difference in the inducibility of AF was also significant after the administration of bepridil [control vs bepridil group =74.0±5.2% vs 24.0±3.3% (day 21), 80.0±5.6% vs 24.0±1.8% (day 28), 88.0±3.5% vs 24.0±7.1% (day 35), and 92.0±2.1% vs 16.0±5.2% (day 42), p<0.05].

When AF was induced at a site in the LA, AF inducibility also increased over the time course, but the inducibility of short-duration AF tended to be higher than that in the RA from day 0; that is, even before starting continuous rapid atrial pacing. In the bepridil group, the sum of the incidence of induced AF was not significantly suppressed compared with the pacing control group, except for day 42 (pacing control group vs bepridil group =100±0.0 vs 66±9.8%, p<0.05), because the inducibility of AF with a shorter duration was not obviously suppressed, but the AF with a long duration (>10 s) gradually decreased. When comparing the 2 groups, the difference in the incidence of inducing AF with a longer duration became significant later than day 28 (pacing control group vs bepridil group =56.0±7.6% vs 16.0±5.3% at day 28, 56.0±7.6% vs 8.0±3.5% at day 35, and 64.0±8.7 vs 6.3±3.5% at day 42, p<0.05).

As can be seen in Fig 4 the duration of induced AF in both atria in the pacing control group gradually increased over the time course. As a result, the mean duration of the induced AF in the RA became longer than that on day 0 on all days later than day 28 (ie, 0.4±2.4 s (day 0) vs 5.7±2.4 s (day 7), 112.7±98.8 s (day 14), 182.0±99.4 s (day 21), NS; 173.7±57.6 s (day 28), 162.5±47.4 s (day 35), and 180.5±51.1 s (day 42), p<0.05). There was a similar tendency in the LA and the mean duration of the AF was prolonged over the time course (ie, 2.4±0.9 s (day 0) vs 50.4±13.7 s (day 14), p<0.01; 71.0±61.7 s (day 21), NS; 195.2±72.2 s (day 28), p<0.05, 246.1±62.9 s (day 35), p<0.01 and 250.8±57.3 s (day 42), p<0.01).

In the bepridil group, the duration of the induced AF in the RA site significantly shortened from the first week after starting drug administration [129.7±44.3 s (day 14) vs 0.8±0.4 s (day 21), 0.4±0.3 s (day 28), 0.6±0.4 s (day 35), and 1.1±0.6 s (day 42), p<0.05]. However, in the LA site, statistical differences were only observed between day 14 and days 35 and 42 (day 14 vs day 35 and 42=28.9±117.7 s vs 2.2±0.5 s and 4.9±1.8 s, respectively, p<0.05). However, when comparing the control and bepridil groups, the differences in both atria between the 2 groups became significant.
from the first week after administration of bepridil [control vs bepridil=182.0±99.4 s vs 0.8±0.4 s (day 21), 173.7±57.6 s vs 0.4±0.3 s (day 28), 162.5±47.4 s vs 6.6±0.4 s (day 35), and 180.5±51.1 s vs 1.1±0.6 s (day 42) in the RA, and 71.0± 61.7 s vs 6.8±2.5 s (day 21), 195.2±72.2 s vs 5.7±0.8 s (day 28), 246.1±62.9 s vs 2.2±0.5 s (day 35), and 250.8±57.3 s vs 4.9±1.8 s (day 42) in the LA, p<0.05]. All episodes of induced AF spontaneously terminated and persistent AF was not induced in this study protocol.

**Ventricular Response Rates**

Fig 5 shows the change in the ventricular response rate during continuous rapid atrial pacing. Because atrioventricular block was not produced during the initial surgery in this model, the ventricular rate was dependent on atrioventricular conduction through the atrioventricular node, which usually exhibited less than 2:1 conduction. Although bepridil has a calcium-channel-blocking effect, there was no significant difference between the pacing control and bepridil groups in the ventricular response rate over the time course. Even within each group, there was no significant difference among the data from each evaluation point over the time course.

**Hemodynamic Parameters**

Table 2 shows the hemodynamic data obtained on day 42 (ie, the end of the protocol). There were no significant differences between the pacing control and bepridil groups in the hemodynamic parameters, including BP, PAP, PAWP and CVP.

**Evaluation of Atrial Tissue Fibrosis**

Fig 6 shows representative examples of the atrial tissue samples from the RA and LA free walls at the end of the protocol in the non-pacing control, pacing control and bepridil groups. Atrial tissue fibrosis is shown in blue by Azan staining. In the pacing control group, the area ratio of the blue-stained area was significantly larger than in the non-pacing group. Bepridil did not affect the ratio of atrial tissue fibrosis. See text for the details. *p<0.05 vs between each group. RA, right atrium; LA, left atrium.

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**Table 2 Comparison of the Hemodynamic Parameters of Each Group**

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<th>Bepridil group</th>
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<td>BP diastole (mmHg)</td>
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<tr>
<td>PA systole (mmHg)</td>
<td>18.0±2.8</td>
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</tr>
<tr>
<td>PA diastole (mmHg)</td>
<td>6.5±1.9</td>
<td>7.0±2.8</td>
<td>NS</td>
</tr>
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<td>PAWP (mmHg)</td>
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<td>CVP (mmHg)</td>
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<td>NS</td>
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<tr>
<td>CO (L/min)</td>
<td>4.2±1.3</td>
<td>6.2±3.2</td>
<td>NS</td>
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</table>

Comparison of hemodynamics was performed at the end of the protocol (ie, 6 weeks after starting continuous rapid atrial pacing). There were no significant differences between the pacing control and bepridil groups.

**BP**, blood pressure; **PA**, pulmonary arterial; **PAWP**, pulmonary arterial wedge pressure; **CVP**, central venous pressure; **CO**, cardiac output.
Azan staining. In the pacing control group, the area ratio of the blue-stained area was significantly larger than in the non-pacing group. Bepridil did not affect the ratio of atrial tissue fibrosis and there was no significant difference between the LA and RA data in this evaluation.

Expression Levels of the mRNAs of the Ion Channel- and Connexin-Related Molecules

Fig 7 shows the expression levels of the mRNAs of the L-type Ca$^{2+}$ channel (LCC), Kv4.3, SCN5A, and connexin (Cx) 40, 43 and 45 evaluated in the samples obtained at the end of the protocol. In comparison with the non-pacing control group, the LCC, Kv4.3, SCN5A and Cx43 were downregulated in the right atrium (RA) of the pacing control group. SCN5A and Cx43 were also downregulated in the left atrium (LA) in the pacing control group, although the changes in the LCC and Kv4.3 were not significant. In the bepridil group, the downregulation of the LCC and SCN5A in the RA was suppressed, but not in the LA. The downregulation of the Kv4.3 and Cx43 was not affected by bepridil administration. See text for the details.

Discussion

Study Design and Results Documenting the Reverse Remodeling Effect of Bepridil on Atrial Electrical Remodeling With Rapid Atrial Pacing

Several clinical reports have documented a cardioversion effect of bepridil on relatively long-lasting persistent AF, and the existence of a “reverse remodeling effect” of bepridil was suspected because the cardioversion effect usually appeared after relatively long-term observation (ie, 2–8 weeks). The term “reverse remodeling” was originally defined as ‘recovery from remodeling as a result of the disappearance or decrease in the stimulation that caused the remodeling’, but the phenomenon caused by bepridil differs from that because the stimulation for the remodeling (ie, AF) continues. However, because there is no other term for expressing this type of recovery under the existence of the stimulation of the remodeling, the term “reverse remodeling” is used to refer to this phenomenon, at least by us.

Previously, we reported the nominative effect of bepridil in a canine 2-week atrial stimulation model, namely, that bepridil suppressed AERP shortening in the first week and further restored the AERP to the pre-pacing level in the second week. As this type of “late recovery” effect on the AERP was not observed when other drugs were subjected to the same evaluation, we also suspected the existence...
of a “reverse remodeling effect” of bepridil. More recently, Nishida et al\textsuperscript{10} clearly documented AERP re-prolongation and recovery of the expression of downregulated LCC expression with administration of bepridil in a study design with an initial 3-weeks of rapid atrial pacing followed by oral administration of bepridil and continuation of the rapid atrial pacing for an additional 3 weeks. They concluded that bepridil had a “reverse remodeling effect”, at least on the shortened AERP and downregulated LCC expression at the mRNA level. In the present study, we also designed a study to test the effect of bepridil on already “remodeled” atrium to mimic downstream therapy for clinical AF by starting the administration of bepridil after an initial 2-week of rapid atrial pacing in a 6-week rapid atrial pacing model in canines. As a result, we reconfirmed the previous study’s main conclusion, but also documented several important differences. First, the degree of recovery of the AERP and expression level of the LCC was similar to that report\textsuperscript{10} in the RA site, but no significant change was observed in the LA site. Second, we documented downregulation of some additional ion-channel- or gap-junction-related molecules that have been suggested as affected by intercellular fibrosis during the structural remodeling\textsuperscript{21,22} (ie, SCN5A, Kv4.3 and Cx43) and importantly, showed that the expression of SCN5A recovered, at least in the RA site, with the administration of bepridil. This is the first report to suggest an effect of bepridil on the expression of SCN5A or Cx.

**Reverse Remodeling Effect of Bepridil**

Considering the results of our study, bepridil had a recovery effect on the shortened AERP and downregulated expression of the LCC and SCN5A in the RA site, even when continuing the rapid pacing, but the decrease in the CV was not affected. In the data from the RA site, a shortened AERP exhibited a relatively quick prolongation in the first week after bepridil administration, and interestingly, that initial prolongation was followed by a gradual recovery of the AERP during the next 3 weeks. Parallel to those changes in the AERP, AF inducibility and duration both decreased, so the changes in the AERP might be related to the anti-AF effect of bepridil. The initial prolongation of the AERP by bepridil was considered to coincide with the increase in plasma and tissue concentrations of bepridil, because bepridil exerts its effect on the AERP via Iks and Ikr blocking effects. In accordance with the results of our previous experimental study\textsuperscript{325} steady-state bepridil concentration was increased, so the changes in the AERP, AF inducibility and duration both decreased, so the changes in the AERP might be related to the anti-AF effect of bepridil. The initial prolongation of the AERP by bepridil was considered to coincide with the increase in plasma and tissue concentrations of bepridil, because bepridil exerts its effect on the AERP via Iks and Ikr blocking effects. In accordance with the results of our previous experimental study, steady-state bepridil concentration was achieved after only 1 week of administration in canines, although a slightly longer period would be necessary in humans. However, to understand the gradual recovery of the AERP in the later weeks, an additional mechanism of bepridil is necessary, so this might be a demonstration of the “reverse remodeling effect” of bepridil on atrial electrical remodeling.

The mechanism of the “reverse remodeling effect” of bepridil is unclear. The effect on the hemodynamic parameters does not explain it because there was no difference between the control and bepridil groups. Recently, Fareh et al reported that a selective T-type calcium-channel blocker, mibebradil, suppressed atrial remodeling with relatively long-term treatment\textsuperscript{18} and Shingagawa et al reported that amiodarone, a multi-channel blocker with a T-type calcium-channel-blocking effect, prevented the downregulation of the LCC in a similar rapid pacing model.\textsuperscript{19} The mechanism is unclear, but a T-type Ca\textsuperscript{2+} blocking effect might play a role in reducing atrial electrical remodeling, because bepridil also has that effect. Re-upregulation of downregulated ion channels, such as SCN5A or LCC, might be a possible mechanism because they may affect the duration of the action potential of the atrial myocardium.

**Effect of Bepridil on the CV and Tissue Fibrosis**

Another important feature of atrial remodeling during AF is the decrease in the CV. According to previous reports, this phenomenon can be explained by downregulation of the sodium channels\textsuperscript{20} redistribution of the Cx family members, and intercellular fibrosis\textsuperscript{21,22}. Because intracellular conduction would be affected by the expression or distribution of the gap junctions, the change in the mRNA expression of the Cx families was also evaluated in this study. Bepridil did not show any suppressive effect on the decrease in the CV or progression of intercellular fibrosis in the present canine experimental model with rapid atrial pacing. Furthermore, bepridil did not affect the expression level of the downregulated Cx43 in either atrium, so although bepridil has an anti-AF effect, suppression of the change in the CV and tissue fibrosis is not the mechanism.

**Inhomogeneity Between the Atria of the Effect of Bepridil**

Another very important result from our study was the difference between the RA and LA sites. In our observation, although AERP re-prolongation and re-upregulation of the LCC and SCN5A were well documented in the RA, the results in the LA were less obvious or insignificant. As a result, although AF with a long duration was suppressed, even in the LA sites, the incidence of inducing medium- or long-duration AF was rather increased in the LA (Fig 3). Therefore, the arrhythmic and reverse remodeling effect of bepridil seemed to be inhomogeneous between the RA and LA. The mechanism of this inhomogeneity is unclear, but residual atrioventricular conduction via the atrioventricular node might have an influence. Although our model protocol did not induce any significant heart failure, even at the end of the study, faster ventricular beats (Fig 5) may result in higher wall stress, especially in the LA free wall\textsuperscript{41} and might cause a more resistant state for the reverse remodeling effect of bepridil. However, because our model (ie, without atrioventricular block) mimicked clinical AF better than a model with atroventricular block, the results of our study should be more compatible with results from clinical cases.

Conversely, the present study did not evaluate the inhomogeneity of the entire atria because the spatial resolution of the evaluating points was limited. Mainly for technical reasons, we limited the evaluation points to just 2 (in the pectinate muscle region in the RA and another in the atrial appendage area in the LA). Because the electrophysiological properties and anatomical structures would differ, especially in the sulcus terminalis area in the RA and pulmonary vein area in the LA, the results might also differ in those areas than is reflected in the results of this study. However, although the results for additional areas of the atria should be evaluated in studies with different designs, our present study has documented different behaviors in representative areas of both atria (ie, at least, the inhomogeneity of atrial electrical remodeling) during administration of bepridil.

**Clinical Implications**

As antiarrhythmic agents are prescribed as a downstream approach in clinical practice, a “reverse remodeling effect” (ie, recovery from remodeling even under continuation of
AF) would be more important as an AF therapy than preventing the initial establishment of remodeling when considering the management of atrial electrical remodeling. As bepridil is a multi-ion channel blocker, it also works as an antiarrhythmic agent, but if it has a "reverse remodeling" effect, this would be a very strong advantage for controlling the arrhythmogenic substrate in AF. Several clinical reports have shown the possibility of using bepridil as a "reverse remodeling" agent, so this effect should be tested in clinical cases. It has been shown that amiodarone, another multi-ion channel blocker, might have a "reverse remodeling effect" in short- and long-term experimental studies; however, the use of amiodarone is limited by its various side-effects. Therefore, bepridil is potentially a more useful antiarrhythmic drug, especially for treating long-lasting AF, without inducing severe side-effects. Nishida et al suggested that bepridil might become a useful alternative to amiodarone for AF therapy and because clinical cases of AF do not have atrioventricular block, our study is considered to more precisely reflect the usefulness and limitations of the use of bepridil in clinical cases.

Study Limitations

First, we did not evaluate the atrial monophasic action potential duration as an index of action potential duration. Because the atrial electrograms were recorded through the sutured epicardial electrodes used in this study, an optimal recording with suitable tension could not be obtained. Second, the evaluation of the CV was totally limited. Because the atrial electrograms were recorded through the sutured epicardial electrodes used in this study, an optimal recording with suitable tension could not be obtained. Finally, the expression of the total atria. Third, the identification of the sampled tissue for histological or mRNA evaluation might have been a problem. Because it is technically impossible to sample the atrial tissue exactly where the epicardial electrodes were sutured, the actual sample was taken from intact atrial tissue close to the electrode. Because the actual distance from the site of the electrode sutting was within 10 mm, the properties of the sampled atrial tissue were considered to be identical to the atrial tissue where the electrophysiological measurement was performed. Finally, the expression levels of the ion channels, ion exchangers or Cx families were evaluated only at the mRNA level, not at the protein or functional level. Therefore, the effect of bepridil on atrial electrical remodeling might differ at those levels, even though the mRNA levels of the LCC, SCN5A or Cx43 were re-upregulated in our observation. These limitations should be solved in future studies with different designs.

References