Ionic Mechanisms of Acquired QT Prolongation and Torsades de Pointes in Rabbits With Chronic Complete Atrioventricular Block

Yukiomi Tsuji, MD; Tobias Opthof, PhD; Kenji Yasui, MD; Yasuya Inden, MD; Haruki Takemura, MD; Noriko Niwa, MD; Zhibo Lu, MD; Jong-Kook Lee, MD; Haruo Honjo, MD; Kaichiro Kamiya, MD; Itsuo Kodama, MD

Background—The ionic basis of acquired QT prolongation and torsade de pointes (TdP) unrelated to drugs is not fully understood.

Methods and Results—We created a rabbit model with chronic complete atrioventricular block (AVB) (n = 34), which showed prominent QT prolongation (by 120%), high incidence of spontaneous TdP (71%), and cardiac hypertrophy. Patch-clamp experiments were performed in left ventricular myocytes from 9 rabbits (8 with TdP, 1 without TdP) at 21 days of AVB and from 8 sham-operated controls with sinus rhythm. Action potential duration was prolonged in AVB myocytes compared with control (61% at 0.5 Hz, 21% at 3 Hz). Both rapidly and slowly activating components of the delayed rectifier K⁺ current (Iₚ and Iₛ) in AVB myocytes were significantly smaller than in control by 50% and 55%, respectively. There was no significant difference in Ca²⁺-independent transient outward current (Iₒ). L-type Ca²⁺ current (Iₖ,L) in control and AVB myocytes was similar in peak amplitude, but the half voltage for activation was shifted to the negative direction (5.9 mV) in AVB myocytes. Voltage dependence of Iₖ,L inactivation was not different in control and AVB myocytes. The inward rectifier K⁺ current (Iₖ) significantly increased in AVB myocytes compared with control.

Conclusions—In the rabbit, chronic AVB leads to prominent QT prolongation and high incidence of spontaneous TdP. Downregulation of both Iₚ and Iₛ in association with altered Iₖ,L activation kinetics may underlie the arrhythmogenic ventricular remodeling. (Circulation. 2002;106:2012-2018.)

Key Words: electrophysiology ■ long-QT syndrome ■ torsade de pointes ■ ion channels ■ action potentials

Bradycardia, including complete atrioventricular block (AVB), may predispose to acquired long-QT syndrome (LQTS) and torsade de pointes (TdP). The dog with chronic AVB has been described as an animal model of acquired QT prolongation and TdP. In the model, the bradycardia-induced volume overload causes biventricular hypertrophy and heterogeneous prolongation of the ventricular action potential. Significant reduction of the slow component of the delayed rectifier K⁺ current (Iₛ) in both ventricles and that of the rapid component (Iₚ) in the RV were demonstrated. TdP was easily induced in the canine model by class III antiarrhythmic drugs (eg, d-sotalol and almokalant) and programmed stimulation, but documented spontaneous TdP episodes and the incidence of sudden cardiac death were relatively rare.

In this study, we have produced a chronic AVB model in the rabbit for the first time. We have assessed arrhythmias in conscious rabbits by the episode-report function of implanted pacemakers. In contrast to the dog, this rabbit model shows a high incidence (71%) of spontaneous TdP during the observation period up to 6 weeks. All rabbits (n = 25) not killed for cellular electrophysiology died suddenly between 0 and 38 days of escape rhythm. Ventricular myocytes isolated from the AVB rabbits were characterized by a prominent action potential prolongation, a significant reduction of both Iₚ and Iₛ, and altered activation kinetics of L-type Ca²⁺ current (Iₖ,L).

Methods

Experiments were performed on Japanese white rabbits (Chubukagaku-Shizai Inc, Japan) weighing 2.0 to 3.0 kg. All rabbits were fed and housed according to institutional guidelines at Nagoya University. This investigation complied with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).
Surgical Technique
Rabbits were anesthetized with a mixture of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg). Suplemental anesthesia was provided by thiamyl sodium (25 mg/kg IV). A right thoracotomy was performed under artificial respiration to induce permanent AVB by injection of 37% formaldehyde into the atrioventricular junction, according to the method applied to rats described by Lee et al.9 During the same session, a unipolar pacing electrode (Medtronic, 6491) was fixed on the free wall of the RV and connected to a pacemaker (Medtronic, Kappa), which was implanted subcutaneously in the back. This pacemaker is equipped with a function to report high ventricular rate episodes. Postoperatively, cefotiam hydrochloride (10 mg/kg) was given for 5 days.

Experimental Protocol
In preliminary experiments, we observed that all of 5 rabbits with AVB but without pacing just after operation died within 24 hours. Therefore, ventricular pacing was performed immediately after creation of AVB at 180 to 200 bpm to allow recovery from surgery. No rabbits died during this period. Five days after operation, the pacing rate was decreased gradually under anesthesia (35 mg/kg ketamine and 5 mg/kg xylazine IM) until escape junctional or idioventricular rhythm appeared. Then pacing was switched off and the limb-leads ECG was recorded as the baseline (day 0) of AVB. A total of 34 rabbits were under escape rhythm for several subsequent weeks. Every week (days 7, 14, and 21), the ECG was recorded after 15 minutes under the same anesthesia and then continuously monitored for ~40 minutes until the rabbit awoke. ECG parameters, including cycle length of an escape rhythm, PP interval, QRS duration, and QT interval, were measured as the average from 3 consecutive beats with a single lead providing the clearest end of the QT interval. Echocardiographic examination was performed at days 0 and 21. The serum electrolytes (Na+, K+, Cl-, Ca2+, Mg2+) measured in 3 rabbits at day 14 were all within normal limits. TdP measured in rabbits at day 14 were all within normal limits. TdP in rabbits at day 14 were all within normal limits. TdP measured in rabbits at day 14 were all within normal limits.

Cellular Electrophysiology
Myocytes were isolated from the apical region of the left ventricle (LV) (subepicardial, midmyocardial, and subendocardial layers were all included).10 Nine AVB rabbits were killed for cell isolation at approximately day 21 of the protocol. Eight sham-operated rabbits with sinus rhythm were used as control.

The membrane potentials and currents were recorded by standard whole-cell patch-clamp method, as reported previously.10 Normal Tyrode’s solution was used as external solution unless otherwise specified, and the bath temperature was maintained at 34°C to 35°C. The internal pipette solution contained 10 mmol/L EGTA (pCa 8.0). Junction potential of the pipette (3 to 5 mV) was 4 to 10 mV (5.6 mV on average). Resting membrane potential recorded by the current clamp mode was corrected for this value. Cell capacitance was determined by applying a ramp voltage pulse of 0.5 V/s at a potential between −50 and +70 mV. The cell capacitance and series resistance (Rs) was compensated for by ~50% to 70%. Action potentials elicited by injecting twice-threshold pulses of 2- to 4-ms duration were analyzed at steady state at each frequency.

When Ik was measured, the myocytes were superfused with Na+-free, K+-free solution including N-methyl-d-glucamine (NMG) to eliminate relatively large inward rectifier K+ current (Ik1). The two components of Ik1, Ik, and Ik2, were pharmacologically separated by the application of a selective blocker, E-4031 (5 μmol/L). The E-4031-sensitive and E-4031-resistant components were measured as indexes of Ik, and Ik2, respectively. The E-4031-resistant component was confirmed as Ik2 by elimination with 30 μmol/L chromanol-293B.10 Ca2+-independent transient outward current (Ito) and Ik1 were measured in Tyrode’s solution including 3 μmol/L nisoldipine. In experiments to measure Ito, KC1 in the external and pipette solution was replaced by CsCl to avoid contamination by K+ currents. For comparison of currents derived from control and AVB myocytes, current amplitudes were normalized to the cell capacitance.

Data Analysis and Statistics
Data are presented as mean±SEM. The pCLAMP program (Axon Instruments) was used in data analysis. Statistical analysis was performed using unpaired Student’s t test and one-way ANOVA.

Results
Rabbit Model With Chronic AVB: ECG Changes
Figure 1A shows an example of an ECG before the AVB creation, immediately (day 0) after the cessation of pacing, and at days 7, 14, and 21 in the same rabbit. Compared with sinus rhythm (SR), rate-dependent QT prolongation was observed at day 0. The QT interval was prolonged at day 7 and more at day 14. The QRS complex also widened as early as after 7 days. An abnormal QTU complex was observed at days 14 and 21. Figure 1B shows a spontaneous TdP at day 14 in this rabbit. Figure 1C summarizes the ECG data of these 5 states. The RR interval increased from 272±20 at SR to

Figure 1. Acquired QT prolongation and TdP in a rabbit with chronic AVB. A. During sinus rhythm (SR) before operation, RR cycle length is 260 ms and QT interval 145 ms. At day 0, RR of escape rhythm is 945 ms. There is rate-dependent QT prolongation to 210 ms. At day 7, QT prolongs to 315 ms with widened QRS complex. At days 14 and 21, it has increased remarkably to 335 and 345 ms at a similar slow rate. B, TdP occurred spontaneously during ECG recording of day 14. C, Time-dependent ECG changes during SR (n=15) before operation and at days 0 (n=34), 7 (n=22), 14 (n=18), and 21 (n=13) during escape rhythm. RR, PP, QRS, and QT times are shown in panels 1 through 4. ▼P<0.05 vs SR; *P<0.05; ††P<0.005 vs day 0.
987±59 ms at day 0 of escape rhythm. It decreased slightly to 799±49 ms at day 7 and more or less stabilized at 846±72 ms at day 21. The PP interval did not change. The QRS duration significantly increased from SR to day 7. Thereafter no additional changes were observed. The QT interval increased from 153±4 ms at SR to 232±6 ms at day 0 and to 302±6 ms at day 7. In contrast to the QRS duration, the QT interval continued to increase after day 7, ultimately to 337±9 ms at day 21.

Spontaneous Episodes of TdP and Survival of Chronic AVB Rabbits

At day 0, a pacemaker was programmed to the OVO mode with a sensing threshold of 1 mV, and the optional function available in Medtronic Kappa was set to detect and store the ventricular high-rate episodes (>8 consecutive beats at >400 bpm) in AVB rabbits. Figure 2A shows 2 examples of stored electrograms. In rabbit 1, polymorphic ventricular tachycardias were documented at day 16. This rabbit with frequent episodes of arrhythmias during day and night (Figure 2B) was killed at day 20. Rabbit 2 had TdP-like ventricular tachycardia at day 3 and frequent episodes before death the next day (Figure 2B).

The spontaneous episodes of TdP were documented in a total of 24 of 34 rabbits (71%). The first TdP stored in the pacemaker varied from day 2 to 21. Average number and duration of the TdP episodes in each animal for the 24 rabbits were 9.9±2.4 and 7.9±3.4 seconds, respectively. Nine of the 34 AVB rabbits (8 with TdP and 1 without TdP) were killed for cell isolation at days 18 through 24. The survival period of the remaining 25 AVB rabbits varied from 0 to 38 days.

Figure 3A shows the relationship between the cycle length of the escape rhythm at day 0 and the survival period of the 25 rabbits not killed. Rabbits with longer cycle length survived over a shorter period. Figure 3B shows the correlation between the date of the first documentation of TdP and survival. There was a linear relationship between the 2 parameters. This suggests that TdP may initiate arrhythmic death.

Echocardiographic Examination

End-systolic and end-diastolic diameter of the LV and right ventricular (RV) diameter increased significantly in AVB rabbits at day 21 compared with those at day 0. The end-systolic wall thicknesses (WTs) of the interventricular septum and of the posterior wall of the LV were increased significantly at day 21, whereas their end-diastolic WTs were unchanged. The fractional shortening of the LV and the left atrial diameter were also unchanged (Table). In 7 of 9 killed rabbits, however, ascites and pleural effusion were observed.
Action Potentials in Myocytes From Rabbits With Chronic AVB

Figure 4A shows representative APs in a control and an AVB myocyte at cycle lengths (CLs) of 333, 1000, and 10 000 ms. The control myocyte typically had a prominent notch in phase 1 at 1000 ms and 10 000 ms, which was preserved in the AVB myocyte. In the AVB myocyte, action potential duration (APD), measured at 50% and 90% of repolarization, was prolonged at each CL. Figure 4B shows the pooled data. The CL/APD90 relationship significantly steepened in AVB myocytes.

The maximal upstroke velocity significantly decreased in AVB myocytes compared with those in control myocytes (92±6 versus 122±9 V/s at 1000 ms CL, *P*<0.05). The resting membrane potentials and the AP amplitudes were not different between AVB and control myocytes. The maximal velocity of repolarization during phase 3 (measured as the most negative first derivative of the membrane potential in that phase) was unchanged. The cell capacitance, an estimate for cell size, in AVB myocytes (172±9.1 pF, *n*=95) was slightly larger than control (163±6.4 pF, *n*=78), but the difference did not reach a statistical significance.

Delayed Rectifier in Control and AVB Myocytes

Figure 5A shows recordings of $I_{K}$, $I_{Kr}$, and $I_{Ks}$ (15 minutes after the membrane rupture). Data from myocytes showing $I_{K}$ reduction >10% between 10 and 15 minutes were discarded to avoid the influence of spontaneous current rundown. The $I_{Kr}$ and $I_{Ks}$ tail current densities were smaller in the AVB myocytes than those in the control myocytes by 50% and 55%, respectively (Figures 5B and 5C). The voltage depen-

### Echocardiographic Findings in AVB Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Day 0 (n=8)</th>
<th>Day 21 (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS, %</td>
<td>64.8±3.7</td>
<td>62.8±4.4</td>
</tr>
<tr>
<td>LV end-diastolic diameter, mm</td>
<td>13.5±0.5</td>
<td>17.2±0.5*</td>
</tr>
<tr>
<td>LV end-systolic diameter, mm</td>
<td>8.2±0.3</td>
<td>10.6±0.6*</td>
</tr>
<tr>
<td>NS diastolic, mm</td>
<td>2.6±0.1</td>
<td>2.6±0.1</td>
</tr>
<tr>
<td>NS systolic, mm</td>
<td>3.7±0.2</td>
<td>4.2±0.2†</td>
</tr>
<tr>
<td>PW diastolic, mm</td>
<td>2.8±0.1</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>PW systolic, mm</td>
<td>4.3±0.1</td>
<td>4.8±0.1†</td>
</tr>
<tr>
<td>LA diameter, mm</td>
<td>9.3±0.4</td>
<td>10.4±0.8</td>
</tr>
<tr>
<td>RV diameter, mm</td>
<td>5.3±0.7</td>
<td>8.3±0.8*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. FS indicates fractional shortening; IVS, interventricular septum; PW, posterior wall of left ventricle; LA, left atrium; and n, number of rabbits.

*P*<0.01, †*P*<0.05, vs day 0.

Figure 4. A, Action potentials recorded in ventricular myocytes from a control and an AVB rabbit. B, Relationship of cycle length (CL) and action potential duration at 90% of repolarization (APD90) in control (CTL) (n=26 from 8 rabbits) and AVB myocytes (n=38 from 9 rabbits). **P*<0.01 vs control.
The voltage dependence of $I_{Ks}$ activation was also unaffected (Figure 5E) ($V_{1/2}$, 2.3 ± 2.5 mV in AVB and 1.2 ± 1.9 mV in control, NS; slope factor, 11.9 ± 1.7 in AVB and 12.4 ± 1.5 mV in control, NS).

**L-Type Ca$^{2+}$ Current**

Figures 6A and 6B show representative recordings and the mean $I$-$V$ relations. The peak amplitude of the $I_{Ca,L}$ density was at 0 mV in control and at −10 mV in the AVB myocytes, but there was no significant difference in the amplitude. Half activation voltage of $I_{Ca,L}$ shifted negatively from −10.6 ± 1.4 mV in control to −16.5 ± 0.7 mV in AVB myocytes ($P<0.01$, Figure 6C). The corresponding slope factor decreased from 4.5 ± 0.7 mV in control to 3.0 ± 0.3 mV in AVB myocytes ($P<0.05$). Voltage dependence of steady-state inactivation was not changed in AVB myocytes. The time constant of $I_{Ca,L}$ inactivation was biexponential. The fast component ($\tau_1$) in AVB myocytes was significantly shorter than in control (at −10 mV, 8.3 ± 1.0 ms in control and 5.4 ± 1.1 ms in AVB myocytes, $P<0.05$, Figure 6D).

**Inward Rectifier K$^+$ Current and Transient Outward Current**

Figure 7A shows representative $I_{Kr}$ recordings (top) and mean current density-$V$ relations (bottom). AVB myocytes exhibited an increased $I_{Kr}$ density compared with control. This increase was larger at test potentials ranging between −50 and −30 mV ($P<0.005$) than at those of −80 and −90 mV ($P<0.05$). There were no differences in either $I_{to1}$ density or inactivation time course (Figure 7B).

**Discussion**

In the present study, we have created an in vivo rabbit model of chronic AVB. We demonstrate that acquired QT prolon-
Downregulation and frequent episodes of spontaneous TdP are associated with downregulation of both $I_{Kr}$ and $I_{Ks}$. There was a negative shift of $I_{Ca,L}$ activation curve in AVB myocytes compared with control. $I_{to1}$ was unaltered, but $I_{K1}$ was increased in AVB myocytes.

High Incidence of TdP in a Rabbit Model With Chronic AVB

In vivo models of chronic AVB have been developed in dogs. In those AVB dogs, reproducible TdP could be induced by pacing with short/long/short sequence or class III antiarrhythmic drugs, but the spontaneous TdP episode and the incidence of sudden cardiac death were relatively low (22% and 10%, respectively). In this study, 24 of 34 AVB rabbits (71%) developed TdP spontaneously, and all of the 25 noneuthanized rabbits died suddenly. The difference of TdP incidence between rabbit and dog models may depend on the degree of QT prolongation (see below).

Our rabbit model shows substantial biventricular hypertrophy in echocardiography with no detectable contractile dysfunction. However, 7 of 9 euthanized rabbits had ascites and pleural effusion, indicating a mixture of compensated and decompensated states. In contrast, most AVB dogs showed compensated biventricular hypertrophy.

Downregulation of $I_{Kr}$ and $I_{Ks}$ in Chronic AVB

In a dog model with chronic AVB, the QT interval was prolonged by 20%. The increase of APD in AVB myocytes was 10% to 30% in the LV (depending on the cycle length) under baseline conditions. There was a 50% reduction of $I_{Kr}$ and no change of $I_{Ks}$ in the LV. This $I_{Kr}$ reduction was considered the cause of APD prolongation in the LV. In the present study, we found a 52% to 120% prolongation of the QT interval and a 20% to 60% increase in APD in the LV myocytes from the rabbit model. The 50% decrease of $I_{Kr}$ in addition to the 55% decrease of $I_{Ks}$ observed in our study may explain the more pronounced prolongation of the AP compared with that in the dog model.

Recently, Emori et al demonstrated in the canine wedge preparation that under conditions of combined $I_{Kr}$ and $I_{Ks}$ block, the transmural ECG shows an extremely large QT prolongation (by 110% to 160%) as well as complex T waves. Our rabbits with downregulation of both $I_{Kr}$ and $I_{Ks}$ also exhibited an abnormal QTU complex in limb leads.

Altered Kinetics of $I_{Ca,L}$ and Increase in $I_{K1}$

In our chronic AVB rabbit model, unlike the dog model, $I_{Ca,L}$ activation curve was shifted to the negative direction by 5.9 mV with no change in the voltage dependence of inactivation, giving rise to an increase of the window current. Although it should be confirmed by single channel recording, such altered voltage dependence of $I_{Ca,L}$ activation would facilitate the generation of early afterdepolarization during the repolarization of action potential. We also observed an increase of $I_{K1}$ density in rabbit AVB myocytes. Recently, an interesting report was published on the difference in rectification of $I_{K1}$ between RV and LV as a determinant of rotor dynamics in ventricular fibrillation in guinea pig heart.

Figure 7. A, Representative $I_{K1}$ recordings and current density. To elicit $I_{K1}$, 300-ms hyperpolarizing or depolarizing voltage steps between -100 and -10 mV were applied at 0.1 Hz from a holding potential of -40 mV. The steady-state currents at the end of the clamp pulses were measured as an index of $I_{K1}$. The $I_{K1}$ density was plotted for AVB myocytes (n=15 from 9 rabbits) and control (CTL, n=8 from 7 rabbits). B, Representative $I_{to1}$ recordings and current density. $I_{to1}$ was elicited with 300-ms depolarizing steps (0.1 Hz) from a holding potential of -50 mV. The $I_{to1}$ density was plotted for AVB myocytes (n=10 from 6 rabbits) and control (n=9 from 6 rabbits). Arrows indicates zero current level. *P<0.05, **P<0.005 vs control.
Implications
The findings of frequent episodes of spontaneous TdP during day and night, the abnormal QTU complex, and the reduction of both $I_{Kr}$ and $I_{KS}$ in this rabbit AVB model closely resemble the clinical characteristics of the malignant form of human congenital LQTS, which is caused by double mutation of HERG and KCNQ1.16 Approaches to molecular understandings of QT prolongation and T-wave abnormalities in this model may additionally underscore the important potential of acquired modification of $I_K$ function in the process of cardiac disease.

Limitation
We cannot specify the exact causes of death in this rabbit AVB model. They could be attributed in part to pump failure, because 7 of 9 euthanized animals showed ascites and pleural effusion. However, some rabbits developed TdP early and died suddenly without signs of heart failure. There was a linear relation between the date of first TdP documentation and survival period, which suggests that TdP initiated arrhythmic death. It would be of interest to clarify when the transition from the compensated to the decompensated state occurs in this model and whether the structural change parallels the process of electrical remodeling.

We investigated the changes in electrophysiological properties in myocytes from the apical region of the LV. Dispersion in repolarization has been recognized as a determinant of TdP in LQTS in many clinical and experimental studies.1 In the dog model with AVB, it has been demonstrated that APD differences between left midmyocardial and right ventricular myocytes cause an enhanced spatial inhomogeneity of repolarization.2 Additional and more extensive experimental studies will be required to clarify interventricular and transmural changes of ionic currents as well as APD in AVB rabbits.

Acknowledgments
We thank Medtronic Japan Co, Ltd for graciously supplying the pacemakers, Dr J.M.T. de Bakker for helpful comments and advice on the manuscript, and K. Matsuo (Medtronic Japan) and T. Suzuki (Japan Lifeline Co, Ltd) for technical assistance.

References
Ionic Mechanisms of Acquired QT Prolongation and Torsades de Pointes in Rabbits With Chronic Complete Atrioventricular Block

Yukiomi Tsuji, Tobias Opthof, Kenji Yasui, Yasuya Inden, Haruki Takemura, Noriko Niwa, Zhibo Lu, Jong-Kook Lee, Haruo Honjo, Kaichiro Kamiya and Itsuo Kodama

_Circulation._ 2002;106:2012-2018; originally published online September 16, 2002; doi: 10.1161/01.CIR.0000031160.86313.24

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/106/15/2012

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the _Permissions and Rights Question and Answer_ document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/