Downregulation of Delayed Rectifier $K^+$ Currents in Dogs With Chronic Complete Atrioventricular Block and Acquired Torsades de Pointes

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Background—Acquired QT prolongation enhances the susceptibility to torsades de pointes (TdP). Clinical and experimental studies indicate ventricular action potential prolongation, increased regional dispersion of repolarization, and early afterdepolarizations as underlying factors. We examined whether $K^+$-current alterations contribute to these proarrhythmic responses in an animal model of TdP: the dog with chronic complete atrioventricular block (AVB) and biventricular hypertrophy.

Methods and Results—The whole-cell $K^+$ currents $I_{T01}$, $I_{K1}$, $I_{Kr}$, and $I_{Ks}$ were recorded in left (LV) and right (RV) ventricular midmyocardial cells from dogs with 9±1 weeks of AVB and controls with sinus rhythm. $I_{T01}$ density and kinetics and $I_{K1}$ outward current were not different between chronic AVB and control cells. $I_{Kr}$ had a similar voltage dependence of activation and time course of deactivation in chronic AVB and control. $I_{Kr}$ density was similar in LV myocytes but smaller in RV myocytes (−45%) of chronic AVB versus control. For $I_{Ks}$, voltage-dependence of activation and time course of deactivation were similar in chronic AVB and control. However, $I_{Ks}$ densities of LV (−50%) and RV (−55%) cells were significantly lower in chronic AVB than control.

Conclusions—Significant downregulation of delayed rectifier $K^+$ current occurs in both ventricles of the dog with chronic AVB. Acquired TdP in this animal model with biventricular hypertrophy is thus related to intrinsic repolarization defects. (Circulation. 1999;100:2455-2461.)

Key Words: electrophysiology ▪ ventricles ▪ torsades de pointes ▪ myocytes ▪ ions

Acquired QT prolongation is a common ECG finding in heart disease, but it can also be present as a primary derangement in the absence of structural abnormalities or drugs. It is regarded as 1 of the risk factors of ventricular arrhythmias and sudden cardiac death. Clinical studies on the acquired long-QT syndromes indicate an enhanced susceptibility to polymorphic ventricular tachycardias, notably torsades de pointes (TdP). Unlike for the congenital long-QT syndromes, in which distinctive defects of ion-channel function in myocardial cells cause delayed repolarization, the ionic basis of acquired QT prolongation not related to drugs is poorly understood.

In recent years, we have used the dog with chronic complete atrioventricular block (AVB) as a model for the study of TdP. QT intervals and ventricular endocardial monophasic action potential durations (APDs) are much longer than expected on the basis of the bradycardia alone and point toward a disturbed ventricular repolarization, corresponding to clinical findings on acquired AVB. Anesthetized dogs show an enhanced susceptibility to acquired TdP after several weeks of AVB duration, which is associated with the development of increased interventricular dispersion of repolarization and early afterdepolarizations. Electrical remodeling is accompanied by the development of biventricular hypertrophy. Approximately 15% of the chronic-AVB dog population dies suddenly, often during circumstances of excitement (eg, during feeding or ambulation). Transmembrane action potential recordings in isolated myocytes indicate that the prolonged ventricular repolarization of chronic AVB is an intrinsic abnormality, which is amplified by class III antiarrhythmic drugs. In addition, action potential prolongation is more pronounced in left (LV) than right (RV) ventricular myocytes, supporting the in vivo finding of increased regional dispersion of repolarization.

In our search for the ionic mechanisms of electrical remodeling and proarrhythmia in dogs with chronic AVB, we investigated the possible contribution of $K^+$-current alterations to ventricular action potential prolongation and in-
creased regional dispersion of repolarization. To this end, we measured whole-cell K⁺ currents in midmyocardial cells of animals with documented TdP and directly compared LV and RV myocytes in the same hearts.

Methods

Animal handling was in accordance with the Dutch Law on Animal Experimentation and the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU). The experiments were approved by the Committee for Experiments on Animals of our university.

In Vivo Studies

Twenty-four adult mongrel dogs of either sex and weighing between 22 and 37 kg were used for the experiments. For a complete description of the creation of AVB (n_
\text{avb}=15), the perioperative care, and the definition of TdP, we refer to a previous publication.²

Anesthesia was induced by sodium pentobarbital 20 mg/kg IV; subsequently, the dogs were ventilated with a mixture of oxygen, nitrous oxide, and halothane (vapor concentration 0.5% to 1.0%). To test the induction of TdP, the class III agent almokalant (0.12 mg/kg IV) was used.² These experiments were performed during anesthesia ≥1 week before the dogs were euthanized for cell isolation.

Cellular Experiments

Anesthetized chronic-AVB (9±1 weeks) and control dogs (with sinus rhythm; n_
\text{controls}=9) received intravenous heparin on thoracotomy. The hearts were quickly excised and washed in cold cardioplegic solution. Heart weights of chronic-AVB dogs were significantly greater than those of control dogs: 306±12 versus 220±8 g, respectively (P<0.05). When corrected for body weight, this difference remained significant: 11.6±0.3 versus 8.6±0.3 g/kg (P<0.05).

To isolate single LV and RV midmyocardial cells simultaneously, the left anterior descending and right coronary arteries were cannulated. The isolation procedure was the same as recently described.⁹ Whole-cell currents were measured with patch pipettes (borosilicate glass) with resistances of 1.0 to 3.0 MΩ when filled with internal solution. Experiments were performed at 37±0.5°C. Cell capacitance was measured by hyperpolarizing steps from a holding potential of −60 mV. In the LV myocytes, average values were 216±9 pF (n_
\text{chronic AVB}=35) versus 227±11 pF (n_
\text{controls}=27; P=NS). In RV myocytes, capacitances were 221±12 pF (n_
\text{chronic AVB}=28) versus 228±11 pF (n_
\text{controls}=29; P=NS). Length times width of these myocytes was, on average, LV Chronic AVB=186×36 μm versus LV Control=193×33 μm (P=NS); RV Chronic AVB=204×35 μm versus RV Control=192×36 μm (P=NS). Compared with a large population study indicating hypertrophy of the individual myocytes in chronic AVB,⁷ the cells used in this study represent the larger bin size.

For the measurements of I_{TO}, we used a holding potential of −70 mV. Na⁺ current was inactivated by a 10-ms prepulse to −40 mV. L-type Ca²⁺ current was inhibited with nifedipine 5 μmol/L. I_{TO} amplitudes were measured as peak amplitudes minus steady-state values at the end of depolarizations. For the measurements of I_{Kr}, I_{Kf}, and I_{Kc}, the holding potential was set at −50 mV. I_{Kr} and I_{Kf} were measured as the peak tail currents on repolarization. For I_{Kc}, we used the tail current sensitive to 2 μmol/L almokalant (specific I_{Kr} blocker) on repolarization to the holding potential of −50 mV. For I_{Kc}, almokalant-resistant tail currents were used.

The standard buffer solution used for the experiments contained (in mmol/L) NaCl 145, KCl 4.0, CaCl₂ 1.8, MgCl₂ 1.0, NaH₂PO₄ 1.0,
were made with ANOVA (for multiple comparisons) and with QT time (measured in ECG lead II) increased from 241 ms (in all chronic-AVB dogs. On average, the QT prolongations in all chronic-AVB dogs. In vivo electrophysiological recordings revealed significant Acquired QT Prolongation and Susceptibility to aspartate 125, KCl 20, MgCl$_2$ 1.0, MgATP 5, HEPES 5, and EGTA 37°C. The patch-pipette solution contained (in mmol/L) potassium glucose 11, and HEPES 10; pH was adjusted to 7.4 with NaOH at 37°C. The patch-pipette solution contained (in mmol/L) potassium aspartate 125, KCl 20, MgCl$_2$ 1.0, MgATP 5, HEPES 5, and EGTA 10C; pH was adjusted to 7.2 with KOH.

The data are expressed as mean±SEM. Intergroup comparisons were made with ANOVA (for multiple comparisons) and with Student’s $t$ test for unpaired and paired data groups, after testing for the normality of distribution. Differences were considered significant if $P<0.05$.

**Results**

**Acquired QT Prolongation and Susceptibility to TdP in the Study Group**

In vivo electrophysiological recordings revealed significant QT prolongations in all chronic-AVB dogs. On average, the QT time (measured in ECG lead II) increased from 241±9 ms at sinus rhythm to 316±14 ms on creation of AVB ($P<0.05$) and to 374±15 ms in the chronic phase at 9±1 weeks thereafter ($P<0.05$ versus acute AVB). Corresponding RR intervals measured 488±24, 1479±142 ($P<0.05$), and 1381±80 ms, respectively (both $P=NS$ versus acute AVB). An example of these 3 states in the same animal is shown in Figure 1. From acute to chronic AVB, QRS widths did not change significantly (see Figure 1). However, broad-based T waves developed early after the QRS complex. In 13 of the 15 animals with chronic AVB, LV and RV endocardial monophasic action potentials were recorded at the idioventricular cycle length. In all 13, the LV APD was longer than the RV APD, on average 419±25 versus 354±19 ms ($P<0.05$), yielding an interventricular difference of 65±12 ms (in line with previous results). In 6 of 7 animals tested with almokalant, TdP occurred.

In the sinus rhythm control group used for comparisons in the cellular investigations, the RR interval was 421±22 ms, with a QT time of 231±6 ms (both $P=NS$ versus preoperative sinus rhythm in the chronic-AVB group).

**I$_{TO1}$ Is Not Altered in Chronic AVB**

The activation of I$_{TO1}$ was tested during steps of $-40$ to $+70$ mV. In all 4 cell types, the current activated at a test voltage ($V_{test}$) $\geq -20$ mV (Figure 2). There was no difference in I$_{TO1}$ density between chronic AVB and control in either ventricle, and the normal interventricular difference was maintained. I$_{TO1}$ was nearly completely inhibited by 5 mmol/L 4-aminopyridine in all 4 cell types. I$_{TO1}$ inactivation during the 300-ms $V_{test}$ was best fitted with a single exponential function yielding similar time constants (range, 7 to 14 ms) in chronic AVB and control. As illustrated in Figures 3A and 3B, voltage dependence of steady-state inactivation and time-dependent recovery from inactivation were not different between the cell types.

**Properties of I$_{K1}$**

Figure 4 shows typical recordings of I$_{K1}$ in chronic-AVB and control myocytes, as well as current density-voltage relations at the end of $V_{test}$ of $-140$ to $-20$ mV in both LV and RV. The current was fully inhibited in K$^+$-free superfusate (0 [K$^+$]; not shown). I$_{K1}$ outward currents at $V_{test}$ positive to the K$^+$ reversal potential were similar in chronic AVB and control. Only at very hyperpolarizing pulses in RV cells were current densities less negative in chronic AVB. There were no interventricular differences between chronic AVB and control.

**Downregulation of I$_{Ks}$ and I$_{Kr}$**

To dissect the 2 components of the delayed rectifier K$^+$ current at baseline, we applied a protocol in which, after a depolarization to $+30$ mV, repolarization was divided into 2 steps: to 0 mV for 4.5 seconds and then back to $-50$ mV (Figure 5). Tail currents at the first repolarization step (to 0 mV) were insensitive to the I$_{Kr}$ blocker almokalant (Figure 5, left arrow in both panels), indicating that they were largely composed of deactivateing I$_{Ks}$ and largely devoid of I$_{Kr}$. Tail currents at the second repolarization step (to $-50$ mV) decreased significantly on almokalant administration, indicating the opposite: namely, that they were composed largely of deactivateing I$_{Ks}$ with a much smaller contribution of I$_{Kr}$ than at 0 mV (Figure 5, right arrow in both panels). From these data, it appeared that I$_{Ks}$ was smaller in chronic-AVB than control myocytes: for LV, 0.19±0.02 versus 0.40±0.09 pA/pF, and for RV, 0.21±0.03 versus 0.51±0.08 pA/pF, respectively (both $P<0.05$). For the same comparison, I$_{Kr}$ was similar in LV, 0.34±0.03 versus 0.42±0.05 pA/pF ($P=NS$), but smaller in RV myocytes, 0.34±0.02 versus 0.48±0.02 pA/pF.
pF, in chronic AVB versus control, respectively (P<0.05). To elaborate further on the contribution of $I_{Ks}$ to total delayed rectifier K$^+$ current, we performed envelope-of-tails tests at baseline and in the presence of almokalant. These experiments revealed that $I_{Ks}$ made up a significant portion of the total current, even for depolarizations as short as 300 ms. However, contributions were much smaller in chronic-AVB than control cells (data not shown).

Voltage-dependent activation of $I_{Ks}$ was evaluated from tail currents on repolarization to $-25$ mV in 0[K$^+$]o in the presence of almokalant. Examples of families of current traces and pooled data are given in Figure 6. There was no saturation of tail-current amplitudes. Voltage-dependence of $I_{Ks}$ activation was similar in the 4 cell types. However, $I_{Ks}$ density was significantly smaller in chronic AVB than in control. Overall, tail currents were reduced by 50% in LV and by 55% in RV myocytes (ie, average percentage of tail-current differences for the conditioning voltages indicated by asterisks [P<0.05] in Figure 6). Whereas interventricular differences of $I_{Ks}$ exist in normal canine hearts (Figure 6), because of the small current amplitudes in chronic AVB, no differences could be discerned. Voltage dependence of $I_{Ks}$ deactivation was not different between chronic AVB and control. Likewise, the time course of deactivation proved similarly fast in all 4 cell types.

$I_{Kr}$ was quantified as the almokalant-sensitive tail-current portion measured by digital subtraction on single-step repolarizations to $-50$ mV in 4.0 mmol/L [K$^+$]o (Figure 7). Activation occurred after depolarizations $\geq -10$ mV and showed saturation at conditioning voltages ($V_{cond} > +20$ mV. Boltzmann fits to the data revealed half points of 0.3±1.1 and $-1.1\pm 3.1$ mV in LV and of $-2.5\pm 2.1$ and 1.1±1.7 mV in RV for chronic AVB and control, respectively (both P=NS). Corresponding slope factors were 5.8±1.0 and 5.7±2.8 mV in LV and 5.5±1.7 and 5.5±1.5 mV in RV (both P=NS). $I_{Kr}$ density was similar in LV myocytes but smaller in RV myocytes of chronic AVB versus control (reduction of 45%; Figure 7). There were no interventricular differences. In both ventricles, voltage dependence and time course of $I_{Kr}$ deactivation were similar for chronic AVB and control.

Discussion

We have demonstrated that acquired QT prolongation in dogs with chronic AVB and documented TdP is associated with significant reductions of $I_{Ks}$ in the LV and RV. In the RV of these animals, $I_{Ks}$ is also downregulated. Of the other K$^+$ currents, $I_{TO1}$ is unaltered, and so is $I_{K1}$ in the physiological range of voltages.

Reproducible Induction of TdP in Chronic AVB

The dog with chronic AVB is a very suitable large-animal model for the study of TdP. In vivo experiments indicate the critical importance of regional dispersion of repolarization, early afterdepolarizations, and multiple ectopic beats for the initiation of TdP.⁸ Interventricular dispersion during arrhythmogenesis probably reflects the existence of significant repolarization gradients in closely adjacent areas, possibly the septum.¹¹ Our most recent results demonstrate that electrical remodeling of the myocardium is the substrate for the enhanced susceptibility to TdP.⁵⁻⁷ The in vivo part of the
present study confirmed that the dogs used for cellular experiments were a representative population with significant QT prolongation and a low threshold for TdP.

**Contribution of Reduced $I_{Ks}$ and $I_{Kr}$ to Action Potential Prolongation**

In a previous study, at serial testing in vivo, it was found that chronic (versus acute) AVB leads to increases of endocardial monophasic APDs of $\approx +30\%$ in the LV and $+20\%$ in the RV. On the basis of our recent microelectrode study, the relative increase of the APD in chronic-AVB myocytes is $10\%$ to $30\%$ in the LV (depending on the pacing cycle length) and maximally $10\%$ in the RV under baseline conditions. In these cells, the class III agents almokalant and $d$-sotalol cause marked action potential prolongation and the occurrence of early afterdepolarizations, which uncovers the importance of $I_{Kr}$ for ventricular repolarization in chronic AVB. Whether a $50\%$ to $55\%$ reduction of $I_{Ks}$ can account for the action potential characteristics in LV and RV can only be answered indirectly. In a recent study on canine LV midmyocardial tissue, the $I_{Ks}$ blocker chromanol 293B at $30\mu mol/L$ increases the APD from $284\pm13$ to $357\pm25$ ms at a pacing cycle length of $2000$ ms ($+25\%$). This concentration reduces $I_{Ks}$ by $\approx 80\%$ in guinea pig myocytes. In a theoretical model of the normal human ventricular action potential, adapted from the Luo-Rudy model, $50\%$ inhibition of $I_{Ks}$ increases the APD from $374$ to $\approx 425$ ms ($+15\%$; Figure 12 of Reference 14). Thus, at rough approximation, the $50\%$ to $55\%$ decrease of $I_{Ks}$ observed in our present study could account for a $10\%$ to $30\%$ action potential prolongation, at least in the LV. The RV exhibits only minor action potential alterations under baseline conditions, despite its additional downregulation of $I_{Ks}$ ($-45\%$). To explain why the alterations are less pronounced in the RV than the LV, we have to consider that the basic determinants of repolarization are different between the 2 ventricles. In the normal canine heart, $I_{To1}$ and $I_{Ks}$ are much larger in the RV, indicating a larger repolarization reserve. In chronic AVB, the difference in $I_{To1}$ is maintained, and it is likely that other membrane currents and/or their remodeling are also responsible for the amplification of interventricular action potential inhomogeneities. We are currently investigating 2 candidates: the L-type Ca$^{2+}$ current and Na$^{+}$-Ca$^{2+}$ exchange.

**Ionic Remodeling in Chronic AVB**

Cardiac function of dogs with AVB of 9 weeks’ duration is unimpaired, which is confirmed at the myocyte level. Most animals have an enhanced contractile performance at the imposed bradycardia and lack signs of heart failure. Significant growth responses (only of cell length) are observed in both RV ($+23\%$) and LV ($+13\%$) myocytes, whereas autopsy findings reveal increased RV and LV tissue weights. These data strongly support the contention that dogs with long-standing (weeks to months) AVB have a compensated form of biventricular hypertrophy.

![Figure 4. $I_{K1}$ in chronic AVB. Examples are from LV myocytes and show currents during $V_{test}$ of $-70$ to $-140$ mV (200 ms; interval 3 seconds). Bottom, Current density-voltage relations for $I_{K1}$ steady-state activation in LV ($n_{Chronic AVB}=8$; $n_{Control}=5$) and RV ($n_{Chronic AVB}=10$; $n_{Control}=8$). In RV, * indicates significant difference ($P<0.05$).](http://circ.ahajournals.org/)

![Figure 5. Activation and deactivation of delayed rectifier K$^+$ current. Activation during a 3-second depolarization to $-30$ mV, followed by deactivation phases at $0$ mV for 4.5 seconds and at $-50$ mV in 2 LV cells (capacitances: chronic AVB, 292 pF and control, 299 pF). In both panels, top current trace is baseline recording, and bottom trace is current during almokalant a few intervals later (interval 20 seconds). Tail currents at first repolarization step (left arrow) are unaltered during almokalant, whereas at second repolarization step (right arrow), they are decreased significantly. In chronic AVB vs control, tail-current amplitudes at first repolarization step are markedly lower in chronic AVB, indicating lower $I_{Ks}$. Similar results were obtained for LV in $n_{Chronic AVB}=8$ and $n_{Control}=11$ and for RV in $n_{Chronic AVB}=11$ and $n_{Control}=16$.](http://circ.ahajournals.org/)
In many other animal models and in humans with cardiac hypertrophy or failure, downregulation of \( K_{\text{1}} \) currents has been implicated in (inhomogeneous) ventricular action potential prolongation\(^{18} \) and the increased risk of ventricular arrhythmias and sudden cardiac death\(^{19} \). Reduction of \( I_{\text{TO1}} \) is probably most often observed in the spectrum of early compensated hypertrophy to terminal heart failure\(^{20} \), and has been linked to action potential prolongation.\(^{21,22} \) However, it has been questioned whether downregulation of this current alone can cause increased APDs in large mammals, including humans.\(^{14} \) \( I_{\text{TO1}} \) downregulation as the basis for action potential prolongation was also challenged by Antzelevitch and coworkers (eg, see Reference \(^{23} \)). Reduction of \( I_{\text{TO1}} \) is clearly absent in dogs with chronic AVB, which corresponds to the finding of marked notch amplitudes in midmyocardial action potentials (RV > LV).\(^{7} \) There have been only a few reports on the downregulation of delayed rectifier K\(^+\) currents in cardiac hypertrophy induced by pressure overload in cats,\(^{24,25} \) but these investigations did not discriminate between \( I_{\text{Kr}} \) and \( I_{\text{Ks}} \). Our data do make this distinction for the hypertrophied cells of dogs with chronic AVB and indicate that changes of these relatively small currents can have major impact on the course of repolarization, as noted before.\(^{26} \)

**Clinical Perspectives**

The importance of \( I_{\text{Ks}} \) and \( I_{\text{Kr}} \) for normal human cardiac repolarization has been established in cellular electrophysiological studies,\(^{27,28} \) and the characteristics of both components resemble those found in the dog. Differential downregulation of RV and LV delayed rectifier K\(^+\) currents could possibly contribute to repolarization abnormalities and arrhythmogenesis in patients with (this or other forms of) cardiac hypertrophy or failure, which is also indicated in a recent modeling of the human ventricular action potential.\(^{14} \) Experimental data on the possible changes of \( I_{\text{Ks}} \) and \( I_{\text{Kr}} \) in human ventricular hypertrophy or failure...
are not yet available, but the importance of these currents can be underscored by the congenital long-QT syndromes.

The combined findings of an enhanced susceptibility to acquired TdP, the (supposed) adrenergic dependence of TdP, the typical T-wave patterns during prolonged QT intervals, and the reduction of \( I_{\text{Kr}} \) in the dog with chronic AVB closely resemble the clinical characteristics of the LQT1 or LQT5 form of the human congenital long-QT syndrome. Approaches to a basic electrophysiological and molecular understanding of QT prolongation and T-wave abnormalities in chronic AVB could be derived from information currently obtained in the long-QT syndrome.

**Limitations of the Study**

We found a differential baseline contribution and hypertrophy-related remodeling of \( K^+ \) currents in the 2 ventricles of the chronic-AVB dog. Similar current alterations could affect the transmural layers of the LV free wall and/or the septum and thus increase local dispersion of repolarization, which would facilitate the induction of TdP. However, this was not investigated.

The studies on \( I_{\text{Kr}} \) were performed with the Ca\(^{2+}\) buffer EGTA in the pipette solution, and thus, Ca\(^{2+}\)-modulated \( I_{\text{Kr}} \) was not recorded. In addition, the response of \( I_{\text{Kr}} \) to stimulation of protein kinase A was not evaluated. The important questions of Ca\(^{2+}\)-dependent and protein kinase A–mediated (dys)function of \( I_{\text{Kr}} \) in chronic AVB are currently being addressed in ongoing studies.

**Conclusions**

Significant downregulation of delayed rectifier \( K^+ \) current contributes to the repolarization abnormalities in the LV and RV of dogs with chronic AVB. The low functional expressions of \( I_{\text{Kr}} \) and \( I_{\text{Ks}} \), in combination with the maintained interventricular difference in \( I_{\text{Kr}} \), and possibly other membrane currents, are responsible for the amplification of interventricular action potential inhomogeneities at control. The resultant increased regional dispersion of repolarization constitutes the substrate for an enhanced susceptibility to acquired TdP.

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