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Analysis of Arrhythmogenic Profile in a Canine Model of Chronic Atrioventricular Block by Comparing In Vitro Effects of the Class III Antiarrhythmic Drug Nifekalant on the Ventricular Action Potential Indices Between Normal Heart and Atrioventricular Block Heart

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Abstract. The chronic atrioventricular block dog is a useful model for predicting the future onset of drug-induced long QT syndrome in clinical practice. To better understand the arrhythmogenic profile of this model, we recorded the action potentials of the isolated ventricular tissues in the presence and absence of the class III antiarrhythmic drug nifekalant. The action potential durations of the Purkinje fiber and free wall of the right ventricle were longer in the chronic atrioventricular block dogs than in the dogs with normal sinus rhythm. Nifekalant in concentrations of 1 and 10 μM prolonged the action potential durations of Purkinje fiber and the free wall in a concentration-dependent manner. The extent of prolongation was greater in the chronic atrioventricular block dogs than in the normal dogs. However, increase of temporal dispersion of ventricular repolarization including early afterdepolarization was not detected by nifekalant in either group of dogs, indicating lack of potential to trigger arrhythmias in vitro. These results suggest that the ventricular repolarization delay in the chronic atrioventricular block model by nifekalant may largely depend on the decreased myocardial repolarization reserve, whereas the trigger for lethal arrhythmia was not generated in the in vitro condition in contrast to the in vivo experiment.

Keywords: action potential, chronic atrioventricular block dog, nifekalant

Introduction

Torsades de pointes is a lethal tachyarrhythmia accompanying long QT interval in the electrocardiogram (ECG), which occasionally appears as an adverse effect during pharmacotherapy (1 – 3). Since such drugs are known to prolong the QT interval by suppression of the rapid component of delayed rectifier K^+ current (I_{Kr}), the International Conference of Harmonization (ICH) S7B guideline for safety pharmacology studies recommends clarification of the effects of developing drugs on cardiac ion channels in vitro and QT intervals in vivo (4). However, a linear correlation has not been established between the drug-induced prolongation of the QT interval and the incidence of torsades de pointes (5). Thus, use of proarrhythmia models such as chronic atrioventricular block animals has been strongly recommended for predicting the future onset of drug-induced long QT syndrome in clinical practice (4, 6 – 11).

Arrhythmogenic substrates in the chronic atrioventricular block dogs have been analyzed by in vitro single cell experiments and in vivo electrophysiological examinations in addition to the anatomical and histological assessments, in which downregulation of K^+

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channels and prolongation of the QT interval together with cellular hypertrophy and interstitial fibrosis were demonstrated (6, 7, 12). Furthermore, trigger of arrhythmias including early afterdepolarization can be spontaneously induced by QT-interval-prolonging drugs via the increase of temporal dispersion of repolarization, leading to generation of ventricular tachyarrhythmias like torsades de pointes (6, 10).

To better understand the arrhythmogenic profile of this proarrhythmia model, in this study, we assessed the action potentials of the isolated ventricular tissues with the intercellular communication preserved, which can provide valuable information concerning the overall electrical activity occurring during the activation/rest cycle (13). We recorded the action potential configuration in the presence and absence of the class III antiarrhythmic drug nifekalant (MS-551) (9, 14, 15) using chronic atrioventricular block hearts and normal hearts. In addition, we investigated the effects of nifekalant on the temporal variability of repolarization by comparing the beat-to-beat variability in the repolarization period, which can clarify the extent of a presage of the trigger for ventricular arrhythmias in the chronic atrioventricular block heart (10, 11).

Materials and Methods

All experiments were performed according to the Guidelines for Animal Experiments, University of Yamanashi and Toho University School of Pharmaceutical Sciences, and comply with the Guiding Principles for the Care and Use of Laboratory Animals Approved by The Japanese Pharmacological Society.

Production of complete atrioventricular block

The 10 beagle dogs employed in these experiments were divided into two groups: 5 atrioventricular block dogs and 5 sham-operated dogs. The surgical procedure using a catheter ablation technique was carried out according to previous reports (7, 11). Briefly, the dogs were anesthetized with pentobarbital sodium (30 mg/kg, i.v.; Tokyo Kasei, Tokyo) and artificially ventilated with room air (SN-408-3; Shinano, Tokyo). The surface lead II ECG was continuously monitored using a polygraph system (RM-6000; Nihon Kohden, Tokyo). A quad-polar electrodes catheter with a large tip of 4 mm (D7-DL-252; Cordis-Webster, Baldwin Park, CA, USA) was inserted through the right femoral vein using the standard percutaneous technique under sterile condition and positioned at the tricuspid valve by watching the bipolar electrograms from the distal electrodes pair. The optimal site for the atrioventricular node ablation, namely, the compact atrioventricular node, was determined on the basis of the intracardiac electrogram, of which a very small His deflection was recorded and the atrium/ventricular voltage ratio was >2. The power source for atrioventricular node ablation was an electrosurgical generator (MS-1500; Mera, Tokyo) delivering continuous unmodulated radiofrequency energy at a frequency of 500 kHz. After proper positioning, in a group of the atrioventricular block dogs, the radiofrequency energy of 20 W was delivered for 10 s from the tip electrode to an indifferent patch electrode positioned on the animal's back, which was continued for 30 s if junctional rhythm was induced. The end point of this procedure was the development of the complete atrioventricular block with an onset of stable idioventricular escaped rhythm. On the other hand, in a group of sham-operated dogs, the radiofrequency energy was not delivered after the detection of the optimal site for atrioventricular node ablation. Proper care was taken for the animals following the experimental period based on our previous report (7).

Cardiac tissue preparation

Two months after the surgery, at which time electrophysiological and anatomical remodeling of the heart was considered to be established (7, 11), the heart was removed from the dogs (8 to 13 kg) under pentobarbital anesthesia (30 mg/kg, i.v.) and artificial respiration. The right ventricular endocardial free wall tissue and septal tissue with Purkinje fibers, which did not show automaticity, were placed in an organ bath containing Krebs-Henseleit solution of the following composition: NaCl 118.4 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 24.9 mM $NaHCO_3$, and 11.1 mM glucose, gassed with 95% O_2 / 5% CO_2 (pH 7.4) at 36 ± 0.5°C.

Microelectrode measurements

The preparations were driven by external electrical stimulation with bipolar platinum electrodes and rectangular current pulses (3- to 5-ms duration, 1.2 – 1.5 × threshold strength) at a constant frequency (1 Hz) generated by an electronic stimulator (SEN-3301, Nihon Kohden). Standard microelectrode penetrations were made into the endocardial surface of ventricular preparations with glass microelectrodes filled with 3M KCl, and action potentials were obtained from cells in the surface layer. The output of a microelectrode amplifier (MEZ-7101, Nihon Kohden) was fed into analyzing systems (Analog-Pro DMA and DSS type IV; Canopus, Tokyo or WIN-CAPA; Physiotech, Tokyo). Action potential parameters of ventricular muscles were overshoot (OS); resting potential (RP); maximum rate of phase 0 depolarization ($\dot{V}_{max}$); and action potential duration at 20%

(APD_{20}), 50% (APD_{50}), and 90% (APD_{90}) repolarization. All experiments were performed at 36.5 ± 0.5°C. Preparations were equilibrated in the bathing solution for at least 45 min before measurements.

Beat-to-beat analysis for temporal dispersion of repolarization

The action potential of 201 consecutive beats under a constant electrical stimulation of 1 Hz was recorded before and after the drug administration. Poincaré plots with $APD_{90}(n)$ versus $APD_{90}(n+1)$ were prepared for each of two analysis time points. The mean orthogonal distance from the diagonal to the points of the Poincaré plot was determined as short-term variability ($=\sum|APD_{90}(n+1) - APD_{90}(n)| / [200 \times \sqrt{2}]$). On the other hand, the mean distance to the mean of the parameter parallel to the diagonal of the Poincaré plot was determined as long-term variability ($=\sum|APD_{90}(n+1) + APD_{90}(n) - 2APD_{90}(mean)| / [200 \times \sqrt{2}]$). These nomenclatures are adopted from heart rate variability investigations using Holter monitoring in humans (16), which have been applied to ventricular repolarization of normal dogs and chronic atrioventricular block dogs (10, 11, 17).

Drugs and chemicals

Nifekalant hydrochloride (M.W. = 441.91) was provided by Nihon Schering (Osaka). The drug was dissolved in distilled water and small aliquots were added to the organ bath to obtain the desired final concentration. All other chemicals were commercial products of the highest available quality.

Statistical analysis

All experimental data are expressed as mean ± S.E.M. To determine statistical significance of differences between atrioventricular block dogs and sham-operated dogs, Student's *t*-test, Student's *t*-test with Welch's correction, or paired *t*-test was used; a value of $P<0.05$ was considered statistically significant.

Results

Comparison of action potential parameters between the sham-operated dogs and chronic atrioventricular block dogs

The action potential configuration of the myocardia

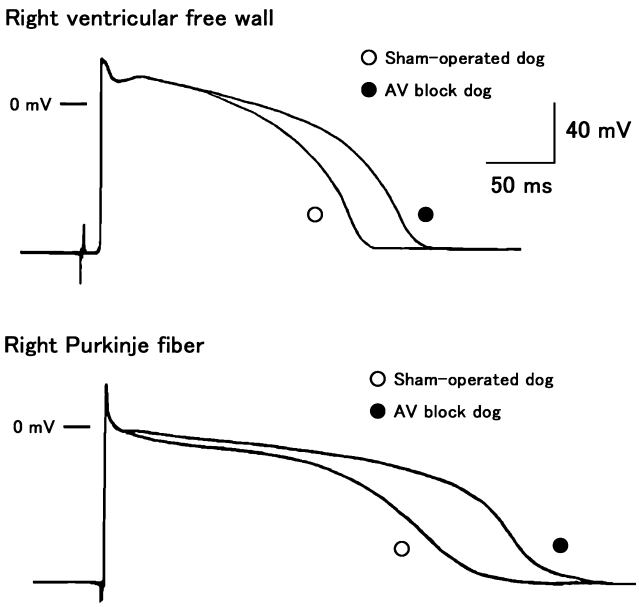

Fig. 1. Comparison of the ventricular repolarization period between sham-operated dogs and atrioventricular (AV) block dogs. Upper and lower panels represent typical tracings of the action potential recorded from the free wall and Purkinje fiber of the right ventricle, respectively. The ventricular tissues were electrically driven at 1 Hz.

Table 1. Comparison of myocardial action potential parameters of the right ventricular tissues between sham-operated and chronic atrioventricular block dogs

	Free wall		Purkinje fiber	
	Sham-operated	AV block	Sham-operated	AV block
APD_{90} (ms)	178.0 ± 2.6	198.3 ± 5.9**	228.3 ± 4.4	253.3 ± 7.9**
APD_{50} (ms)	139.0 ± 3.4	146.6 ± 6.4	152.1 ± 5.3	171.5 ± 8.0
APD_{20} (ms)	82.1 ± 2.8	82.1 ± 4.5	7.5 ± 1.2	4.8 ± 1.0
RP (mV)	–74.8 ± 0.7	–73.4 ± 0.8	–80.4 ± 0.9	–79.8 ± 0.6
OS (mV)	24.4 ± 0.8	27.1 ± 1.1	26.1 ± 1.6	25.6 ± 1.7
AMP (mV)	99.1 ± 1.0	100.5 ± 1.6	106.5 ± 1.3	105.3 ± 1.5
$\dot{V}_{max}$ (V/s)	152.9 ± 7.1	162.6 ± 13.6	285.4 ± 13.4	309.3 ± 16.9

Data are each expressed as the mean ± S.E.M. of 18 – 38 experiments. $APD_{20}/APD_{50}/APD_{90}$, action potential duration at 20%, 50%, and 90% repolarization, respectively; RP, resting potential; OS, overshoot; AMP, amplitude; $\dot{V}_{max}$, maximum rate of phase 0 depolarization. **$P<0.01$, compared with the sham-operated animal group.

from atrioventricular block dogs and sham-operated dogs had myocardial action potentials with similar characteristics. However, the resting potential, overshoot, and $\dot{V}_{max}$ might be underestimated (18, 19) because the endocardial surface of the myocardium in beagle dogs, unlike those of mongrel dogs, was covered with a layer of fibroadipose tissue that might have hampered smooth penetration of microelectrodes into

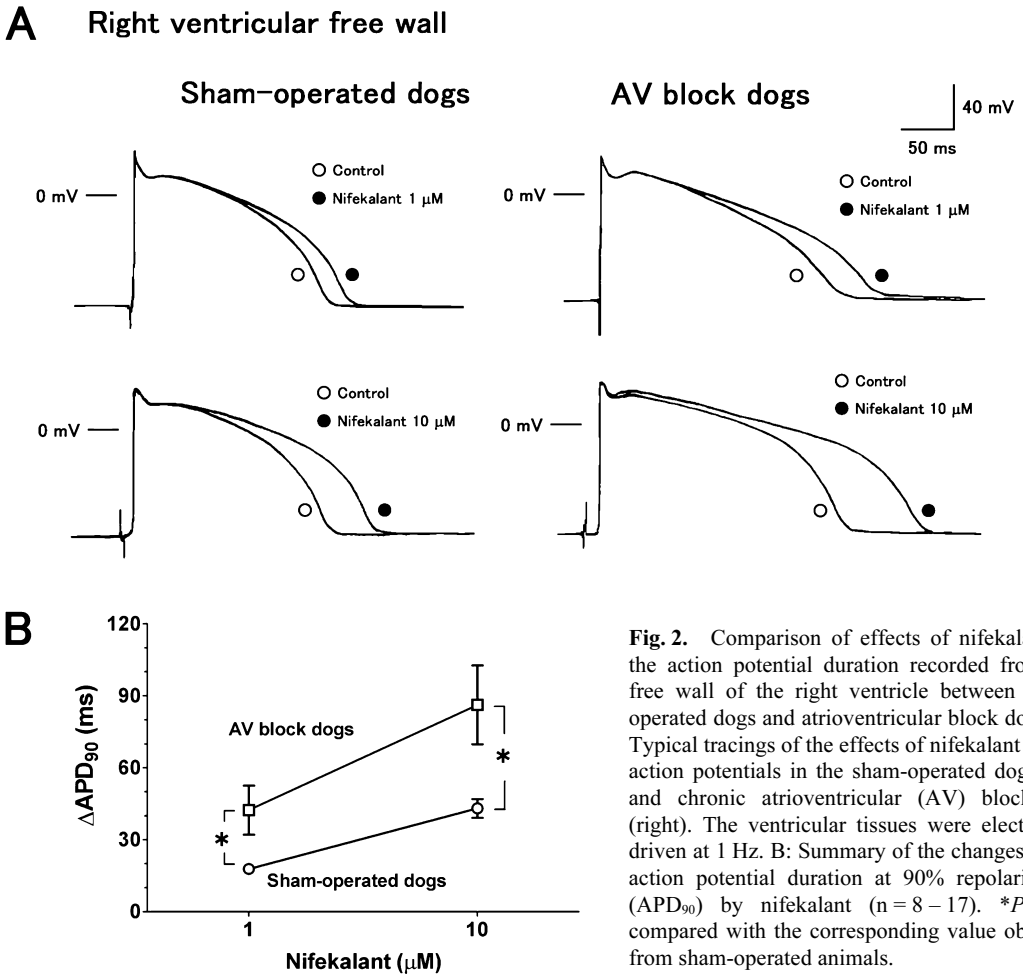

Fig. 2. Comparison of effects of nifekalant on the action potential duration recorded from the free wall of the right ventricle between sham-operated dogs and atrioventricular block dogs. A: Typical tracings of the effects of nifekalant on the action potentials in the sham-operated dog (left) and chronic atrioventricular (AV) block dog (right). The ventricular tissues were electrically driven at 1 Hz. B: Summary of the changes in the action potential duration at 90% repolarization (APD_{90}) by nifekalant (n = 8 – 17). *P<0.05, compared with the corresponding value obtained from sham-operated animals.

Table 2. Effects of nifekalant on the action potential parameters of the free wall of the right ventricle isolated from the sham-operated and chronic atrioventricular block dogs

	Nifekalant (1 μM)		Nifekalant (10 μM)	
	Pre	Post	Pre	Post
Sham-operated				
APD_{90} (ms)	178.1 ± 4.8	193.9 ± 4.7***	174.2 ± 3.8	217.3 ± 5.4***
APD_{50} (ms)	131.9 ± 7.2	148.5 ± 5.2**	138.7 ± 4.4	170.8 ± 5.8***
APD_{20} (ms)	77.1 ± 5.6	77.8 ± 6.2	81.3 ± 4.3	86.2 ± 4.4
AV block				
APD_{90} (ms)	195.6 ± 6.7	237.4 ± 12.8**	201.9 ± 13.4	288.1 ± 16.4**
APD_{50} (ms)	146.9 ± 6.6	175.3 ± 8.3**	150.0 ± 14.9	216.1 ± 18.8**
APD_{20} (ms)	82.5 ± 5.5	86.1 ± 5.7	84.3 ± 9.9	99.3 ± 9.8

Data are each expressed as the mean ± S.E.M. of 8 – 17 experiments. Nifekalant was perfused for 60 min. APD_{20}/APD_{50}/APD_{90}, action potential duration at 20%, 50%, and 90% repolarization, respectively. **P<0.01, ***P<0.001, compared with the corresponding pre-value.

the underlying myocardium, which could form a barrier between the reference electrode and the extracellular surface of the cardiomyocyte. The APD_{90} was longer in the atrioventricular block dogs than in the sham-operated dogs both in the free wall and Purkinje fiber of the right ventricle (Fig. 1 and Table 1). No significant difference was observed in other parameters between the atrioventricular block dogs and the sham-operated dogs.

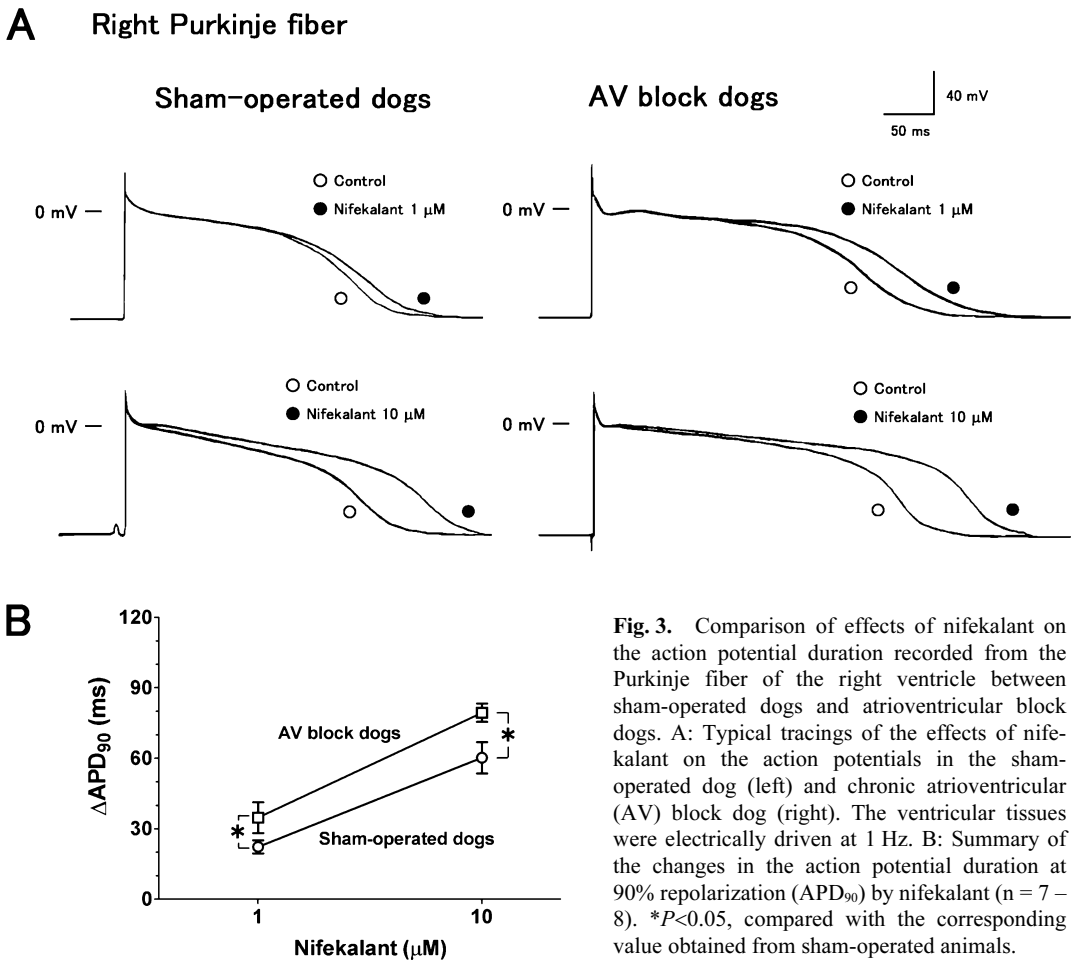

Fig. 3. Comparison of effects of nifekalant on the action potential duration recorded from the Purkinje fiber of the right ventricle between sham-operated dogs and atrioventricular block dogs. A: Typical tracings of the effects of nifekalant on the action potentials in the sham-operated dog (left) and chronic atrioventricular (AV) block dog (right). The ventricular tissues were electrically driven at 1 Hz. B: Summary of the changes in the action potential duration at 90% repolarization (APD_{90}) by nifekalant (n = 7 – 8). *P<0.05, compared with the corresponding value obtained from sham-operated animals.

Table 3. Effects of nifekalant on the action potential parameters of the Purkinje fiber of the right ventricle isolated from the sham-operated and chronic atrioventricular block dogs

	Nifekalant (1 μM)		Nifekalant (10 μM)	
	Pre	Post	Pre	Post
Sham-operated				
APD_{90} (ms)	232.8 ± 6.0	256.4 ± 8.0***	229.9 ± 7.9	289.3 ± 12.0***
APD_{50} (ms)	153.7 ± 7.9	167.0 ± 9.7*	162.2 ± 6.4	205.4 ± 11.1***
APD_{20} (ms)	8.8 ± 2.5	15.6 ± 8.0	5.5 ± 0.8	8.7 ± 2.1
AV block				
APD_{90} (ms)	263.6 ± 15.2	305.3 ± 18.3***	262.0 ± 18.1	343.6 ± 17.2***
APD_{50} (ms)	185.1 ± 16.2	221.6 ± 22.2**	175.1 ± 18.2	240.2 ± 13.4***
APD_{20} (ms)	3.8 ± 0.7	3.9 ± 0.8	3.7 ± 1.0	6.3 ± 3.9

Data are each expressed as the mean ± S.E.M. of 7 – 8 experiments. Nifekalant was perfused for 60 min. APD_{20}/APD_{50}/APD_{90}, action potential duration at 20%, 50%, and 90% repolarization, respectively. *P<0.05, **P<0.01, ***P<0.001, compared with the corresponding pre-value.

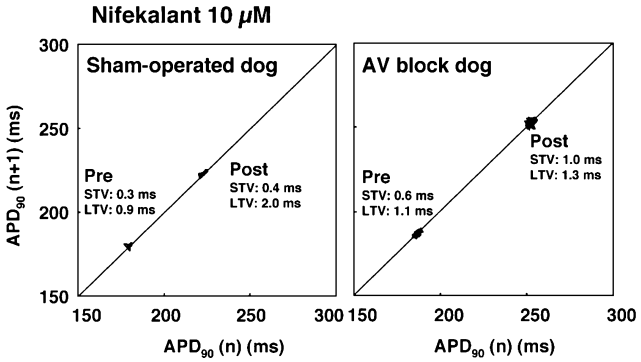

Fig. 4. Poincaré plots of the action potential duration at 90% repolarization (APD_{90}) of the free wall of the right ventricle obtained from the sham-operated dog (left) and chronic atrioventricular (AV) block dog (right). Two hundred one beats were plotted for each of 2 analysis time points: before (pre) and 60 min after 10 μM of nifekalant administration (post). STV: short-term variability and LTV: long-term variability.

Comparison of effects of nifekalant on the action potential parameters between sham-operated dogs and atrioventricular block dogs

Nifekalant (1 and 10 μM) concentration-dependently prolonged the APD_{90} and APD_{50} at both regions in the atrioventricular block dogs and in the sham-operated dogs (Figs. 2A and 3A and Tables 2 and 3). The prolongation of APD_{90} observed in the free wall was significantly longer in the atrioventricular block dogs than in the sham-operated dogs (Fig. 2B). Similarly, the prolongation of APD_{90} observed in Purkinje fiber was significantly longer in the atrioventricular block dogs than in the sham-operated dogs (Fig. 3B). The early afterdepolarization was not detected in the sham-operated or atrioventricular block dogs before or after the treatment of nifekalant.

Beat-to-beat analysis for the temporal dispersion of repolarization

As shown in Fig. 4, beat-to-beat analysis was employed for the free-wall tissues with 10 μM nifekalant administration. The action potential of 201 consecutive beats was recorded under the electrical stimulation at 1 Hz. As summarized in Table 4, nifekalant increased the APD_{90} without affecting short-term variability or long-term variability in either animal group.

Discussion

Since the clinically effective plasma concentration of nifekalant has been reported to be around 0.8 μg/ml (=2 μM) in patients with inducible sustained ventricular arrhythmias (20), nifekalant in concentrations of 1 and 10 μM as used in this study reflects therapeutic and supra-therapeutic levels, respectively. As shown in the present study, prolongation of the action potential duration by nifekalant was greater in the isolated myocardium from the chronic atrioventricular block dogs than the sham-operated animals. Meanwhile, increase of temporal dispersion of repolarization; namely, a series of marker of trigger for ventricular arrhythmias, was not detected in the isolated chronic atrioventricular block dog heart even after the treatment with 10 μM of nifekalant, which has been reported to induce early afterdepolarization in the isolated normal rabbit heart (14), suggesting that the isolated heart of this canine model may be less sensitive to the K^+ channel blocker nifekalant for generation of the trigger for lethal arrhythmia than the normal rabbit heart.

Table 4. Effects of nifekalant on the temporal variability of repolarization of the right ventricular free wall tissue isolated from the sham-operated and chronic atrioventricular block dogs

	Nifekalant (1 μM)		Nifekalant (10 μM)	
	Pre	Post	Pre	Post
Sham-operated				
APD_{90} (ms)	188.2 ± 8.6	207.2 ± 7.5***	176.2 ± 2.6	218.4 ± 9.7*
STV (ms)	0.6 ± 0.2	0.6 ± 0.3	0.5 ± 0.1	0.5 ± 0.1
LTV (ms)	1.8 ± 0.7	1.2 ± 0.4	0.9 ± 0.2	1.5 ± 0.3
AV block				
APD_{90} (ms)	216.1 ± 18.7	241.7 ± 23.0	234.0 ± 32.8	301.3 ± 36.5***
STV (ms)	0.6 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.6 ± 0.2
LTV (ms)	1.4 ± 0.4	1.2 ± 0.4	1.4 ± 0.2	1.7 ± 0.3

Data are each expressed as the mean ± S.E.M. of 3 – 5 experiments. APD_{90}, action potential duration at 90% repolarization; STV, short-term variability of repolarization; LTV, long-term variability of repolarization. *P<0.05, ***P<0.001, compared with the corresponding pre-value.

Action potential parameters in the chronic atrioventricular block dogs

As shown in Fig. 1 and Table 1, the action potential duration of the isolated myocardium was longer in the chronic atrioventricular block dogs than sham-operated animals, which is essentially in accordance with previous electrophysiological studies using the isolated ventricular cardiomyocytes and the right ventricle in vivo under the constant cardiac pacing (7, 21). In previous electrophysiological studies using the right ventricular cardiomyocytes of chronic atrioventricular block dogs, downregulation of the slow component of delayed rectifier K^+ current (I_{Ks}) and I_{Kr} channels was detected, whereas L-type Ca^{2+} channels were intact (12, 22), which may explain the longer action potential duration in this study.

Extent of the repolarization reserve in the chronic atrioventricular block heart

Nifekalant prolonged the action potential duration both in the Purkinje fiber and the ventricular free wall in a concentration-dependent manner. Nifekalant has been reported to suppress the delayed rectifier K^+ current (I_K) at 3 μM, transient outward current (I_{to}) at 10 μM, and inward rectifier K^+ current (I_{K1}) at 10 μM in the rabbit isolated ventricular cells (14), which may explain the nifekalant-induced ventricular repolarization delay in this study. As described in the results, the extent of the prolongation of the action potential duration was greater in the atrioventricular block dogs than the sham-operated animals. The density of I_{Ks} channels in the ventricular cardiomyocyte of the chronic atrioventricular block dogs has been reported to decrease to about half of that of normal animals (12). Since previous electrophysiological studies demonstrated that pharmacological blockade of I_{Ks} enhances the I_{Kr} blocker-induced action potential prolongation via the reduction of repolarization reserve (23 – 25), the enhanced susceptibility to QT interval prolonging drugs in the chronic atrioventricular block dogs may largely depend on decrease of the repolarization reserve.

Lack of increment of temporal variability of repolarization by nifekalant

Beat-to-beat variation of the ventricular repolarization period has been shown to precede occurrence of the early afterdepolarization in the isolated ventricular cardiomyocyte from chronic atrioventricular block dogs (10). Thus, the temporal variability of repolarization, which can be estimated from the short-term variability of ventricular repolarization period, is now considered to be a reliable predictive maker for drug-induced torsades de pointes arrhythmias in the chronic atrioventricular block animal model (10, 11). In this study, the pre-drug value of the short-term variability of repolarization was 0.6 – 0.7 ms in the isolated chronic atrioventricular block heart, which is not as large as that observed in our previous in vivo study using chronic atrioventricular block dogs (around 5 ms) (11). Furthermore, the temporal variability of repolarization was hardly affected by 10 μM nifekalant (Table 4), a concentration that prolonged the action potential duration by +28%. In contrast, in our previous in vivo study, oral administration of nifekalant to the canine chronic atrioventricular block model induced torsades de pointes when the corrected QT interval was prolonged by 20% (9). Because plasma catecholamine levels have been reported to be significantly elevated in the chronic atrioventricular block model (6, 26), increase of adrenergic tone would be essential in generation of the trigger for lethal arrhythmias (6 – 11).

Conclusion

These results suggest that the repolarization delay in the chronic atrioventricular block model by nifekalant may largely depend on the decreased myocardial repolarization reserve, whereas the trigger for lethal arrhythmia was not generated in the in vitro condition in contrast to the in vivo experiment (9).

Acknowledgments

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