3.2. Family 2

Family 2 is a case of CPVT appeared in monozygotic twins. The older sister is 14-years-old girl and since 11-years of age recurrent syncope occurred after exercise. No structural abnormality was detected and VT or VF was not induced by programmed electrical stimulation (PES) at EP study. Treadmill stress test induced VT in a reproducible manner. Infusion of Isoproterenol (0.01 mg/min) induced NSVT and Epinephrine infusion(1 mg/min) induced PVT followed by VF. Implanted caridoverter defibrillator (ICD) was implanted and oral β-blocking therapy initiated. ICD discharged appropriately during emotional stress. The younger sister is 14-years-old girl with a history of bronchial asthma. Syncope appeared after exercise and emotional stress since 13-years-old. No structural abnormality was detected but treadmill stress test induced VT in a reproducible manner. VT or VF was not induced by PES at EPS study. BVT and PVT appeared following ISP infusion (0.01 mg/min). ICD was implanted and calcium blocker therapy initiated. ICD discharge occurred repeatedly and calcium blocker was replaced by a β-blocker. Direct sequences of these patients identified C to T transition at the position of 6737 leading to amino acid change of S for L 2359: S2246L. Since their parents do not have this mutation, de novo mutation was suggested.

3.3. Family 3

The patient was a 53-years-old female, who suffered from recurrent syncope during exercise since 30-years of age. She had family history of sudden deaths in 4 family members over fourth generations. No structural abnormality was detected and VT was not induced by the programmed electrical stimulation at EP study. Treadmill stress test induced multiple PVCs following by PVT. ICD implant was rejected and β-blocking agent suppressed symptoms during 2 years of follow up. We performed genetic analysis against this family after written informed consent was obtained. Direct sequences of the patients identified G to A transition at the position of 7076 and T to G transition at 14552 leading to amino acid change of R for Q 2359 (R2359Q) and F for C 4851 (F4851C).

4. Summary and conclusion

We identified 3 mutations of RyR2 in 4 CPVT families (75%) but none in other arrhythmic disorders. All of the previously reported mutations are missense mutations locating in three hot lesions; N-terminus, calstabin 2 binding region and pore. It is well known these mutations clustered similar regions of the MH/CCD mutations of RyR1, suggesting the functional importance of these regions in both RyR1 and RyR2. The FKBP12.6 KO mice died after exercise followed by epinephrine injection due to polymorphic ventricular tachycardia and ventricular fibrillation [4]. It is suggested that exercise activates PKA and phosphorylates RyR2 channel and result in dissociation of FKBP12.6 and RyR2. Dissociation of FKBP12.6 and RyR2 produce leaky Ca-channels during diastole, which trigger delayed after depolarizations (DADs) and cardiac arrhythmias.

In conclusion, to prevent sudden cardiac death in childhood and adolescence, early diagnosis of CPVT is of critical importance. The *RyR2* mutations were highly observed in patients with CPVT but not other ion channel disorders. Existence of CPVT related *RyR2* mutation is known to be associated with early onset and the genetic study against CPVT patients is of critical importance.

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Multiple Premature Beats Triggered Ventricular Arrhythmias During Pilsicainide Infusion in a Patient with Inferior ST-Segment Elevation

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A 17-year-old man was referred to our hospital for treatment of common paroxysmal atrial flutter. His electrocardiogram at rest showed subtle ST-segment elevation in leads II, III, and aV_F. Intravenous pilsicainide caused further ST-segment elevation in the inferior leads, new ST-segment depression in leads V2–V6, two distinct forms of premature ventricular complexes (PVCs) triggering short runs of polymorphic ventricular tachycardia (VT). An infusion of isoproterenol suppressed these arrhythmias and normalized the ST-segment. Pilsicainide may induce PVCs and polymorphic VT in atypical Brugada syndrome. (PACE 2006; 29:1445–1448)

atypical Brugada syndrome, polymorphic ventricular tachycardia, pilsicainide

Introduction

Brugada syndrome is characterized by ST-segment elevation in leads V1–V3 and development of ventricular fibrillation (VF).[1,2] However, some patients with an atypical (or inferior) form of the syndrome develop ST-segment elevation in the inferior leads, in addition to typical changes on the surface electrocardiogram (ECG).[3–5] We report observations made in a young man whose ECG showed ST-segment elevation in leads II, III, and aV_F. Intravenous pilsicainide caused further ST-segment elevation and induction of two types of premature ventricular complexes (PVCs), which triggered short runs of polymorphic ventricular tachycardia (VT).

Case Report

A 17-year-old man was referred to our hospital for management of the common type of paroxysmal atrial flutter. The arrhythmia was successfully treated by radiofrequency catheter ablation, which induced conduction block between the tricuspid valve annulus and inferior vena cava. The patient had no personal history of syncope or cardiac arrest, and no family history of sudden death. Examination of his cardiovascular system was normal. The standard 12-lead ECG showed sinus rhythm and subtle ST-segment elevation in leads II, III, and aV_F (Fig. 1A), and late potentials were present on signal-averaged ECG (RMS40 = 17.9 μV, LAS40 = 46 ms, and f-QRS = 118 ms).

A pharmacological challenge test with intravenous pilsicainide, 40 mg over 4 min, was performed, which caused further ST-segment elevation in leads II, III, and aV_F, along with the development of ST-segment depression in leads V2–V6 (Fig. 1B). These ECG changes were associated with multiple PVCs with two distinct QRS morphologies. A first morphology was of left bundle branch block (LBBB) and superior axis (Fig. 2A), and the other of LBBB and normal axis (Fig. 2B). The coupling interval of the PVCs was between 440 and 460 ms, coinciding with the end of the T wave of the preceding cycle. Soon thereafter, short runs of polymorphic VT were repetitively triggered by both forms of PVCs (Fig. 2A, B). The PVCs and polymorphic VT were both suppressed, and the ST-segment normalized, by the intravenous administration of isoproterenol (Fig. 1C). The patient remained asymptomatic throughout the pilsicainide test.

Cardiac catheterization and electrophysiologic studies, including programmed ventricular stimulation, were performed a few days later. The coronary angiogram and left ventriculogram were normal. The His-ventricular (HV) interval during sinus rhythm was prolonged to 65 ms. VF was twice induced by triple right ventricular (RV), apical extrastimulation. The patient and his family declined implantation of a cardioverter defibrillator and genetic analysis of the cardiac ion channels. At 22 months of follow-up, the patient has remained free of adverse cardiac events on no specific medical therapy.

Discussion

Except for the presence of ST-segment elevation in the inferior leads of the ECG, this patient presented with findings consistent with Brugada

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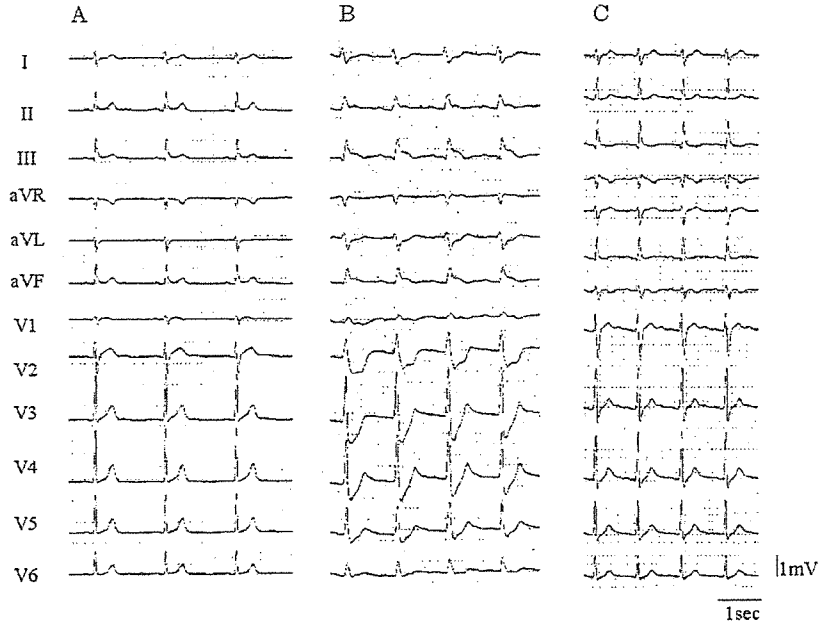

Figure 1. *(A) Baseline 12-lead ECG. Subtle ST segment elevation is present in leads II, III, and aV_F. (B) Pilsicainide accentuated the inferior ST-segment elevation and caused ST-segment depression in leads V2–V6. (C) Isoproterenol infusion normalized the ST-segment in both the inferior and anterior leads.*

syndrome. ST-segment elevation was accentuated by the intravenous administration of pilsicainide, (a sodium channel blocker), and normalized by the infusion of isoproterenol.[1,2] Along with the increase in ST-segment elevation, two different forms of PVCs were induced, which, in turn, triggered short runs of polymorphic VT.[6]

ST-segment elevation in Brugada syndrome has been attributed to different action potential configurations in the RV endocardium versus epicardium, creating a prominent transmural dispersion of ventricular repolarization.[7,8] Since I_{to} channels are predominantly distributed in the epicardial region of the RV outflow tract, ST-segment elevation, in Brugada syndrome, is typically present in leads V1–V3. However, several cases have been reported of Brugada-type ST-segment elevation in leads II, III, and aV_F (atypical or inferior Brugada syndrome), also attributed to prominent transmural dispersion of ventricular repolarization through the inferior ventricular wall.[3–5]

Unlike previous reports of atypical Brugada syndrome,[3–5] our patient developed ST-segment depression in leads V2–V5 and concomitant ST-segment elevation in the inferior leads in response to the administration of pilsicainide. Similar ECG changes might sometimes be observed during acute myocardial ischemia caused by right coronary artery occlusion. However, myocardial ischemia was unlikely in this case, since our patient was young, asymptomatic during the ECG changes, and had normal coronary angiograms and left ventriculogram. Furthermore, intravenous pilsicainide is unlikely to induce coronary vasospasm, since sodium channels are known to be present neither in vascular smooth muscle nor in endothelial cells. However, in some patients with Brugada-type ECG abnormalities, an abnormal vascular expression of sodium channels or the indirect release of vasoconstrictors by pilsicainide might exist. Indeed, vasospastic angina could coexist with Brugada syndrome,[9] and has been reported following the administration of pilsicainide.[10] In addition to Brugada-like ST-segment elevation in the inferior leads and pilsicainide-induced PVCs and polymorphic VT, our patient had atrial flutter, prolongation of the HV interval, and an abnormal signal-averaged ECG. He might be the carrier of a SCN5A gene mutation, which has been described in some patients with Brugada syndrome. However, he and his family did not consent to further investigations. Another possibility of the ST-segment depression in this patient might be due to accentuation of the action potential notch and/or depression of the action potential plateau in the endocardium secondary to the presence of a more prominent I_{to} in this tissue.

It is noteworthy that two types of PVCs with LBBB pattern developed following ST-segment

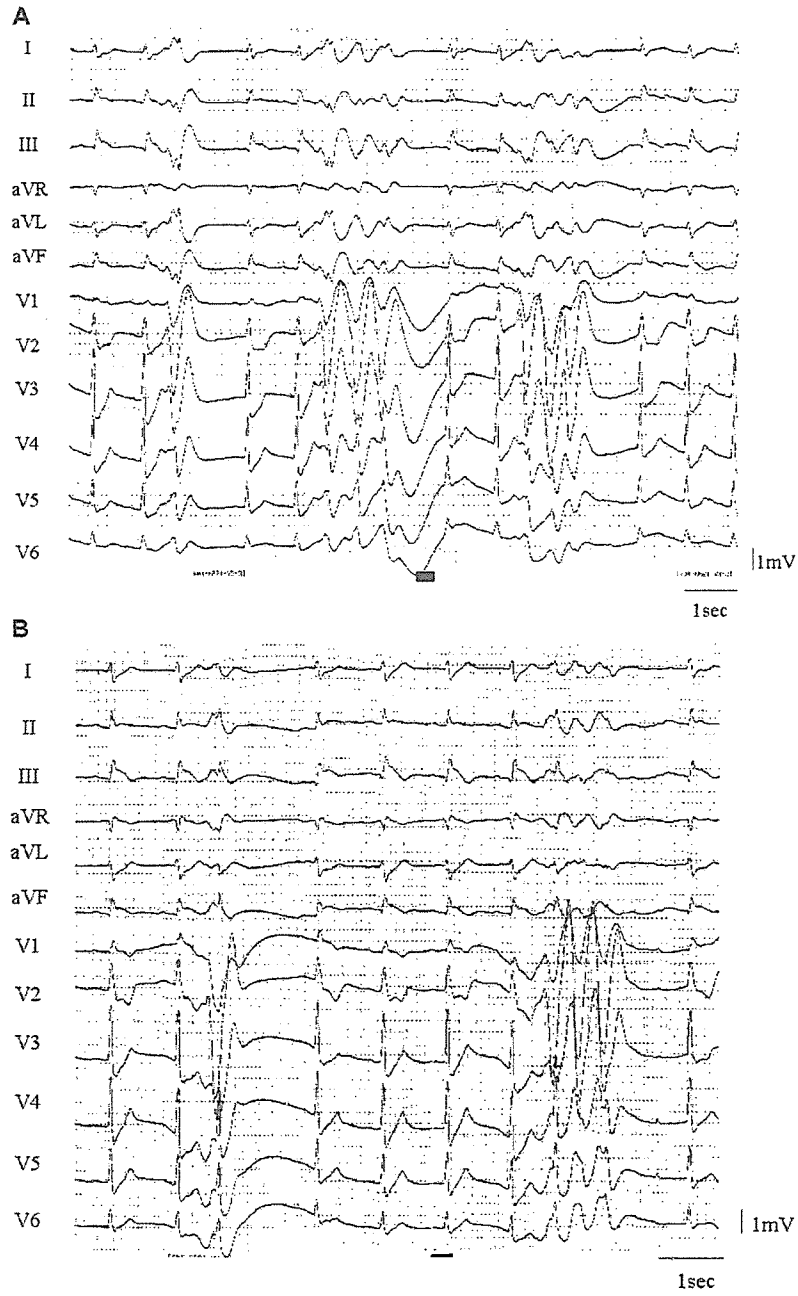

Figure 2. *ECGs recorded during the pilsicainide test. Frequent PVCs with LBBB morphology and superior axis (A), or LBBB morphology and normal axis (B) triggered short-runs of polymorphic VT.*

elevation induced by pilsicainide, both of which triggered short-runs of polymorphic VT. The initiation of VF in Brugada syndrome has been attributed to phase 2 reentry between endocardial and epicardial layers, or between two RV epicardial regions,[7,8] and the triggered PVCs might originate from epicardial areas with exaggerated I_{to} currents and shorter action potential duration. Therefore, the multifocal PVCs observed in this patient suggest that the epicardial myocardium with exaggerated I_{to} current was not confined to a small RV region.

Although the mechanism of inferior ST-segment elevation in our patient seems uncommon and has not been clarified, class I anti-arrhythmic drugs should be used with caution in this clinical context since they may induce ventricular arrhythmias, or coronary vasospasm, or both.

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Role of Autonomic Nervous Activity in the Antiarrhythmic Effects of Magnesium Sulfate in a Canine Model of Polymorphic Ventricular Tachyarrhythmia Associated with Prolonged QT Interval

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Abstract: This study was performed to examine the role played by the autonomic nervous system in the antiarrhythmic effects of magnesium sulfate (Mg^{++}) in a canine model of polymorphic ventricular tachyarrhythmia facilitated by anthopleurin-A and a slower heart rate induced QT interval prolongation. In 6 experiments, complete atrioventricular block was created to control the heart rate and bradycardia at 800- to 1500-ms cycle lengths was applied for 60 sec before and after drug-induced autonomic block. Transmural unipolar electrograms were recorded from multipolar needle electrodes, and activation-recovery intervals (ARI) were measured. Before drug-induced autonomic block, polymorphic ventricular tachyarrhythmia developed in all 6 experiments during bradycardia before but not after the administration of Mg^{++} (0.2 ml/kg intravenous bolus). During drug-induced autonomic block, triggered premature activity decreased without significant changes in underlying dispersion of repolarization and polymorphic ventricular tachyarrhythmia developed during bradycardia in 1 experiment. Administration of Mg^{++} during drug-induced autonomic block eliminated premature activity and polymorphic ventricular tachyarrhythmia during bradycardia. The distribution of left ventricular (LV) and right ventricular repolarization and dispersion of transmural repolarization were analyzed before and 60 sec after Mg^{++} administration during ventricular pacing at 80 bpm. Mg^{++} caused a modest shortening of ARI at all sites before and after drug-induced autonomic block. Since ARI shortening was greater at the mid-myocardial sites than at other LV sites, Mg^{++} decreased transmural ARI dispersion from 77 ± 16 to 46 ± 21 ms before drug-induced autonomic block and from 79 ± 7 to 51 ± 16 ms after drug-induced autonomic block. The antiarrhythmic effects of Mg^{++} in this model of long QT syndrome were attributable to its direct pharmacological properties and not to changes in ambient autonomic nervous activity. The blockade of sympathetic activity decreased the incidence of premature events and partially suppressed polymorphic ventricular tachyarrhythmia in this model.

Key Words: magnesium therapy, autonomic nerve activity, autonomic blockade, prolonged QT interval

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INTRODUCTION

Magnesium sulfate (Mg^{++}) is a common treatment of polymorphic ventricular tachycardia developing in association with QT interval prolongation.[1,2] However, the mechanisms behind the electrophysiologic and therapeutic effects of Mg^{++} have not been thoroughly studied. We recently reported that the antiarrhythmic effect of Mg^{++} in a canine anthopleurin-A model of long QT syndrome (a model resembling type 3 long QT syndrome) was associated with the suppression of triggered premature activity and attenuation of transmural dispersion of left ventricular (LV) repolarization.[3] However, the decrease in systemic blood pressure caused by intravenous Mg^{++} might enhance sympathetic nervous activity,[3,4] which might in turn potentiate the antiarrhythmic effects of Mg^{++} in the experimental model.

We used a new experimental protocol to study the electrophysiological effects of Mg^{++} and role of the autonomic nervous system in the canine model of long QT syndrome. Transmural electrograms were recorded from the left and right ventricles, and the modulation of the ventricular repolarization and antiarrhythmic effects of Mg^{++} against polymorphic ventricular tachyarrhythmia were observed before and after drug-induced autonomic block.

METHODS

Surgical Preparation

This study was approved by the Animal Studies Subcommittee of our Institutional Review Board and was in compliance with the guidelines of the United States National Institutes of Health for the Care and Use of Laboratory Animals. The experiments were performed in 6 beagles weighing between 9.0 and 13.0 kg, anesthetized with a 17.5-mg/kg bolus of sodium thiopental (intravenous) followed by

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a maintenance dose of 5.0 mg/kg/h, and intubated and artificially ventilated. Catheters were inserted into the femoral vein for administration of fluids and drugs and into the femoral artery to monitor blood pressure. The core temperature was kept at 37° C with a thermostatically controlled thermal blanket. Leads I, III, and aV_F of the surface electrocardiogram and blood pressure were continuously monitored. The heart was exposed via midline sternotomy, and saline warmed to 37° C was regularly applied to moisten the heart and prevent cooling of the epicardial surface. Upon completion of the experiments, the animals were killed by electrical induction of ventricular fibrillation under general anesthesia.

Recording and Pacing Electrodes

Four 21-gauge, stainless steel, plunge needle electrodes were inserted in the basal region of the lateral LV wall, where prominent M cell–like activity has been described in the dog,[5] and 3 plunge electrodes were inserted in the right ventricular (RV) free wall. Each LV needle had 8 and each RV 6 polyimide-coated tungsten wire electrodes (diameter, 50 μm; spacing, 1 mm) for simultaneous transmural recording of unipolar electrograms from epicardial (Epi), mid-myocardial (Mid) and endocardial (Endo) sites, with the last electrode located approximately 0.5 mm under the epicardial surface. The last plunge electrode recorded the Epi electrogram, and the first electrode recorded the Endo electrogram. The electrode that recorded the longest activation-recovery interval (ARI) between Epi and Endo was representative of the Mid ventricular layer. ARI was measured as the time interval between the minimum first derivative of the intrinsic deflection of the QRS and maximum first derivative of the T wave of the unipolar electrograms.[6] In previous studies in this model, ARI from unipolar electrograms closely approximated the local effective refractory period, regardless of the T wave morphology.[7]

Complete atrioventricular block was produced by radiofrequency catheter ablation of the atrioventricular node, and the heart was paced from bipolar silver wire electrodes inserted in the RV, with 2.0 ms pulses delivered by a programmable cardiac stimulator at twice the diastolic threshold.

Electrogram Acquisition and Measurements of Activation-Recovery

In each experiment, 50 transmural unipolar electrograms (4 LV needles × 8 electrodes + 3 RV needles × 6 electrodes) were amplified and filtered at a fixed high-pass setting of 0.05 Hz and 500-Hz adjustable low-pass setting. Analog data were digitized at a 1000-Hz sampling rate (F-tech, Niigata, Japan). The digitized signals were stored in the memory of a personal computer. ARI was measured at each unipolar electrogram, and transmural ARI dispersion was measured as the widest difference in ARI among 8 LV or 6 RV unipolar electrograms from each needle electrode. Since 7 needle electrodes were inserted in each experiment, 42 data points (7 needles × 6 experiments) were available for the statistical analysis of ARI and its dispersion. Interventricular ARI difference was calculated as the widest difference between LV and RV ARI in each experiment.

Pharmacologic Interventions

Anthopleurin-A, dissolved in sterile saline, was administered as a 5-μg/kg intravenous bolus, followed by a maintenance dose of 0.15 μg/kg/min. Mg^{++} (0.5 mol/l) was injected in intravenous boli of 0.2 ml/kg. Drug-induced autonomic block was achieved by the intravenous administration of 0.04 mg/kg atropine and 0.2 mg/kg propranolol.

Study Protocol and Data Collection

After creation of complete atrioventricular block, the heart was paced at 100 bpm from the RV during the procedure of experimental setting, and anthopleurin-A was administrated before starting the following study protocol. First, the cardiac cycle was lengthened to 800 to 1500 ms for 60 sec during demand back-up RV pacing at 40 bpm. If polymorphic ventricular tachyarrhythmia developed, its initial characteristics were examined from the transmural ventricular electrograms. The same experimental steps were repeated immediately after the intravenous administration of Mg^{++}, and its antiarrhythmic effect against polymorphic ventricular tachyarrhythmia was observed.

Second, recordings of ARI and of its transmural dispersion and interventriclar difference were obtained before and 60 sec after the administration of Mg^{++} during RV pacing at 80 bpm. The same measurements were made during drug-induced autonomic block with atropine and propranolol. Average values of ARI and ARI dispersion were calculated from 3 consecutive cycles.

Third, the heart rate was slowed again during demand back-up RV pacing at 40 bpm, as described above, during drug-induced autonomic block to evaluate the role played by the autonomic nervous system in the antiarrhythmic activity of Mg^{++}.

The experimental protocol was completed within 3 hours after creation of atrioventricular block (about 2.5 hours after starting anthopleurin-A administration).

Statistical Analysis

Values are presented as means ± SD. Statistical comparisons of ARI and percent shortening of ARI by Mg^{++} infusion among the 3 layers of the ventricle (Endo, Mid, and Epi) and the interventricular ARI alteration by Mg^{++} infusion were made by 2-factor analysis of variance (ANOVA) with repeated measurements (SAS version 8, SAS Institute, Cary, NC). The ARI and percent shortening of ARI difference between 2 of the 3 layers of the ventricle (Mid vs. Epi, Mid vs. Endo, and Endo vs. Epi) were assessed using the Scheffe's test. For statistical analysis of the effect of Mg^{++} administration to the transmural ventricular, ARI dispersion were performed using 3-factor ANOVA with repeated measurements. $P < 0.05$ was considered statistically significant.

RESULTS

Polymorphic Ventricular Tachycardia and Mg^{++} Administration

After creation of atrioventricular block by radiofrequency ablation, escape rhythm at cycle lengths between

800 and 1500 ms was allowed for 60 sec during back-up ventricular pacing at 40 bpm. Multiple premature ventricular events and sustained polymorphic ventricular tachyarrhythmia requiring cardioversion developed in all 6 experiments (Figure 1A). As previously reported,[7,8] the onset of polymorphic ventricular tachyarrhythmia was associated with the development of triggered premature events, which, as they propagated, caused delayed conduction, conduction block, or both at Mid/Endo LV sites, where the longest ARI were recorded during baseline rhythm. Delayed conduction or conduction block was infrequently recorded in the RV (Figure 1A). After the infusion of Mg^{++}, the same slow heart rate for 60 sec did not induce triggered premature events or polymorphic ventricular tachyarrhythmia in any experiment (Figure 1B).

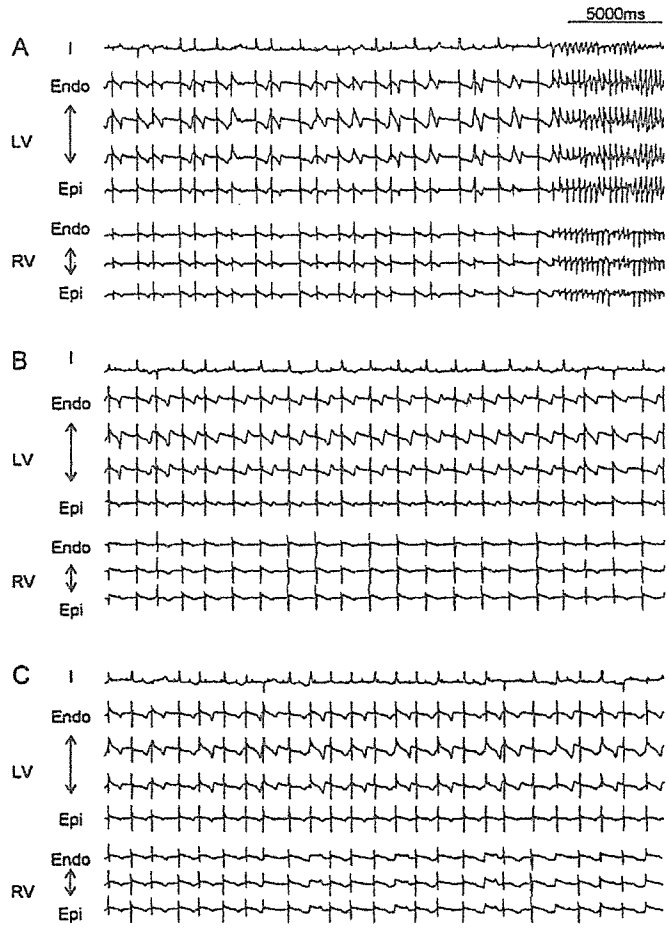

FIGURE 1. Antiarrhythmic effects of magnesium sulfate (Mg^{++}) before and after drug-induced autonomic block. A lead I of the surface electrocardiogram and selected transmural unipolar electrograms from the left and right ventricles. At baseline (A), during bradycardia, multifocal ventricular events developed, one of which triggered polymorphic ventricular tachycardia. Mg^{++} suppressed the premature activity and polymorphic ventricular tachycardia during bradycardia (B). Drug-induced autonomic block decreased the premature ventricular activity without apparent change in the underlying dispersion of repolarization (C). See text for additional details. Endo, endocardial site; Mid, mid-myocardial site; Epi, epicardial site.

After administration of atropine and propranolol, the ventricular escape cycle was mildly prolonged to 1000 to 1500 ms during back-up pacing at 40 bpm, and the number of premature ventricular events was decreased (Figure 1C). Slowing of the heart rate for 60 sec induced polymorphic ventricular tachyarrhythmia in 1 of the 6 experiments. After administration of Mg^{++}, neither premature ventricular events nor polymorphic ventricular tachyarrhythmia was observed in any experiment during bradycardia for 60 sec.

Transmural and Interventricular Ventricular Repolarization

Before Drug-Induced Autonomic Block

The changes in ventricular repolarization induced by the administration of Mg^{++} were examined during ventricular pacing at 80 bpm, a rate chosen because ventricular or junctional rhythms escaping after the creation of atrioventricular block were consistently less than 80 bpm. Before Mg^{++} administration, ARI at the LV Endo, Mid, and Epi sites measured 487 ± 19 ms, 519 ± 21 ms, and 442 ± 25 ms, respectively (Table 1, Figure 2). ARI at the LV Mid site was significantly longer than at the Epi and Endo sites, with a wide ARI dispersion (77 ± 16 ms) created across the LV wall (Table 2). ARI in the Mid RV site (470 ± 21 ms) was slightly longer than at the RV Epi (434 ± 21 ms) and Endo (449 ± 17 ms) sites (Table 3, Figure 3), creating a smaller ARI dispersion (35 ± 10 ms) across the RV than across the LV wall (Table 2). The interventricular ARI difference between LV and RV was 121 ± 21 ms.

The injection of Mg^{++} shortened ARI at all sites (Figures 2 and 3; Tables 1 and 3). Since the magnitude of ARI shortening by Mg^{++} was significantly greater at the Mid than at the other LV sites (−12 ± 5% at Endo, −14 ± 4% at Mid, −10 ± 5% at Epi; $P = 0.0079$ by ANOVA), the transmural ARI dispersion at 60 sec after Mg^{++} administration had decreased to 46 ± 21 ms (Table 2). In the RV, the magnitude of ARI shortening by Mg^{++} was similar in each layer of the ventricle (−10 ± 4% at Endo, −11 ± 4% at Mid, −9 ± 5% at Epi; $P = 0.3005$ by ANOVA), and transmural ARI dispersion at 60 sec after Mg^{++} administration was 27 ± 14 ms. After Mg^{++} administration, the interventricular ARI difference decreased to 87 ± 9 ms, while the systolic/diastolic blood

TABLE 1. Activation-recovery Intervals in Left Ventricle

	ENDO	MID	EPI	*P* for Layers
Autonomic block (−)				
Baseline	487 (19)*	519 (21)*	442 (25)*	<0.0001
Mg^{++}	422 (31)	445 (32)	399 (34)	<0.0001
P for Mg treatment	0.0004	0.0003	0.0051	
Autonomic block (+)				
Baseline	501 (19)*	527 (23)*	448 (27)*	<0.0001
Mg^{++}	437 (29)	458 (29)	410 (30)*	<0.0001
P for Mg treatment	0.0003	0.0003	0.0059	

Mean (SD).
*$P < 0.05$ vs. another two layers.

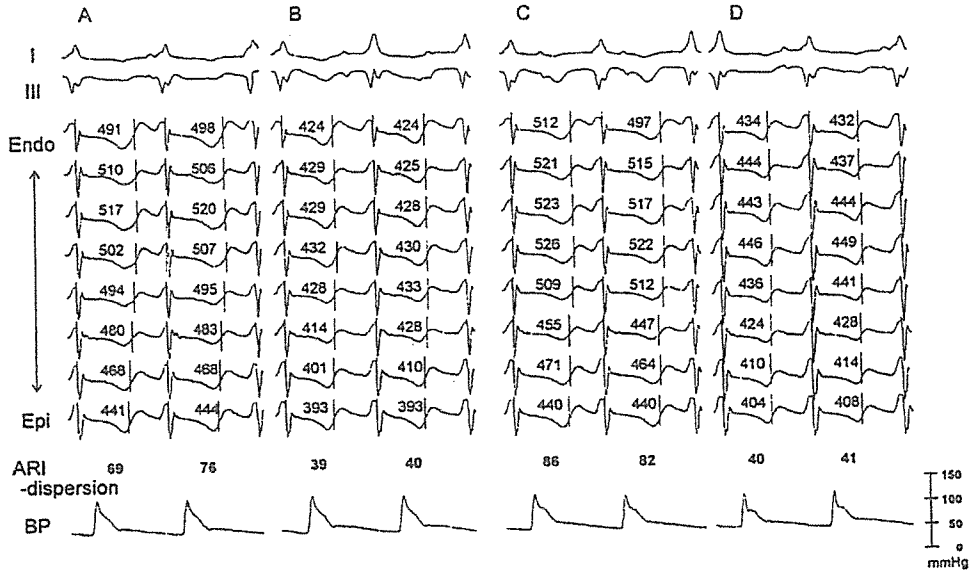

FIGURE 2. Transmural electrograms in the left ventricle before and after Mg^{++}. Before drug-induced autonomic block (A), ARI at the Mid sites is longer than at the Epi/Endo sites, and a large ARI dispersion (69 to 76 ms) is present. ARI shortened at all sites after administration of Mg^{++} (B), and ARI dispersion decreased to 39 to 40 ms. After drug-induced autonomic block, the effects of Mg^{++} on transmural ventricular repolarization were similar, and ARI dispersion decreased from 82 to 86 ms (C) to 40-41 ms (D). The cardiac cycle was fixed at 750 ms by ventricular pacing. Vertical lines in each beat indicate the minimum first derivative of the QRS complex and the maximum first derivative of the T wave. ARI-dispersion, dispersion of activation-recovery interval; BP, systemic blood pressure; Endo, endocardial site; Mid, mid-myocardial site; Epi, epicardial site.

pressure decreased slightly from 122 ± 33/52 ± 12 mmHg to 117 ± 28/48 ± 14 mmHg.

During Drug-Induced Autonomic Block

Drug-induced autonomic block prolonged ARI slightly, and the administration of Mg^{++} again shortened ARI at all sites (Tables 1 and 3; Figures 2 and 3). ARI shortened in all layers of both ventricles, most prominently in the LV (Tables 1 and 3). Since the magnitude of ARI shortening was significantly greater in the Mid layer of the LV (−12 ± 3% at Endo, −13 ± 4% at Mid, −9 ± 3% at Epi; $P < 0.0001$ by ANOVA), transmural ARI dispersion in the LV decreased from 79 ± 7 at baseline to 51 ± 16 ms at 60 sec after administration of Mg^{++} (Table 2). In the RV, the magnitude of ARI shortening by Mg^{++} was similar in each layer (−9 ± 4% at Endo, −10 ± 3% at Mid, −9 ± 3% at Epi; $P = 0.3156$ by ANOVA), such that the change in transmural ARI dispersion after Mg^{++} administration, albeit significant, was considerably less prominent than in the LV. Interventricular ARI difference decreased from 127 ± 23 ms to 88 ± 22 ms, while systolic/diastolic blood pressure decreased slightly from 111 ± 34/46 ± 11 mmHg to 104 ± 38/41 ± 9 mmHg between before and 60 sec after the administration of Mg^{++}.

The magnitude of ARI shortening by Mg^{++} before and after drug-induced autonomic block was similar at all sites in both ventricles (Tables 1 and 3). There was no difference in the effects of Mg^{++} administration to the transmural ARI dispersion and to the interventricular ARI difference before and after attempting drug-induced autonomic block.

TABLE 2. Transmural Dispersion of Activity-recovery Intervals

	Autonomic Block (−)	Autonomic Block (+)	*P* for Autonomic Block
Left ventricle			
Baseline	77 (16)	79 (7)	0.3726
Mg^{++}	46 (21)	51 (16)	
P for Mg treatment	0.0003		
Right ventricle			
Baseline	35 (10)	39 (6)	0.2969
Mg^{++}	27 (14)	30 (18)	
P for Mg treatment	0.0311		

Mean (SD).

TABLE 3. Activation-recovery Intervals in Right Ventricle

	ENDO	MID	EPI	*P* for Layers
Autonomic block (−)				
Baseline	449 (17)	470 (21)*	434 (21)	<0.0001
Mg^{++}	404 (19)	419 (22)*	396 (16)	0.0009
P for Mg treatment	0.0008	0.0012	0.0016	
Autonomic block (+)				
Baseline	464 (28)	487 (26)*	448 (26)	<0.0001
Mg^{++}	427 (32)	438 (31)	409 (15)	<0.0001
P for Mg treatment	0.0066	0.0022	0.0017	

Mean (SD).
*$P < 0.05$ vs. another two layers.

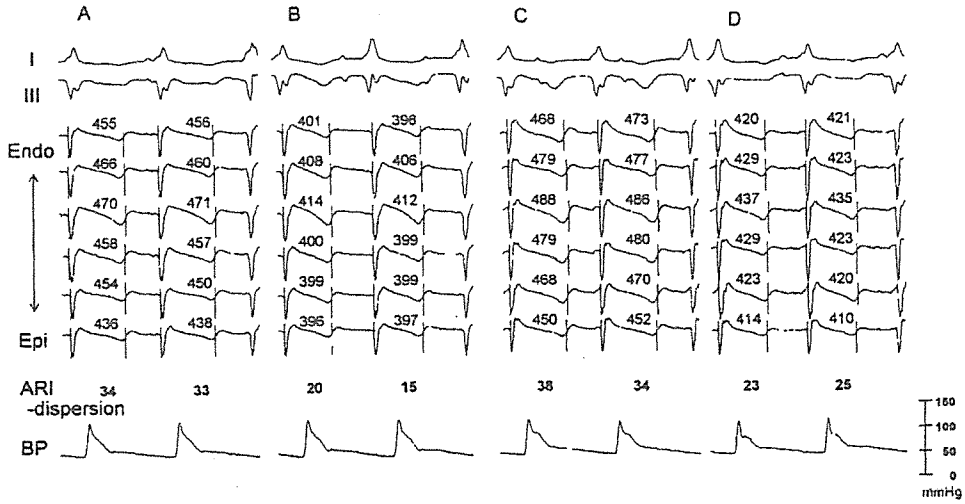

FIGURE 3. Transmural electrograms in the right ventricle before and after Mg^{++}. Before drug-induced autonomic block (A), ARI at the Mid sites was slightly longer than at the Epi/Endo sites and ARI dispersion was 33 to 34 ms. ARI shortened at all sites after the administration Mg^{++} (B), and its ARI dispersion decreased to 15 to 20 ms. After drug-induced autonomic block, the effects of Mg^{++} on transmural ventricular repolarization were similar, and ARI dispersion decreased from 34 to 38 ms (C) to 23 to 25 ms (D). The cardiac cycle was fixed at 750 ms by ventricular pacing. Vertical lines in each beat indicate the minimum first derivative of the QRS complex and the maximum first derivative of the T wave. Endo, endocardial site; Mid, mid-myocardial site; Epi, epicardial site.

DISCUSSION

This study had two main findings. First, the suppression of triggered premature events and homogenization of ventricular repolarization by intravenous Mg^{++} in this model of long QT syndrome was associated with the direct pharmacological properties of Mg^{++} and was weakly influenced by drug-induced autonomic block. Second, the suppression of triggered premature ventricular events partially suppressed polymorphic ventricular tachyarrhythmia.

Autonomic Nervous Activity

In this and previous experiments, 3 intravenous Mg^{++} decreased the systemic blood pressure slightly but might sufficiently activate the sympathetic nervous system. We therefore hypothesized that, in this model, the shortening and homogenizing of ventricular repolarization by Mg^{++} was not primarily attributable to its direct pharmacological properties. Sympathetic nerve activity increases the net outward current in myocytes through the I_{ks} channel, an effect apparent in models of type 3 long QT syndrome in contrast to type 1 or type 2 long QT syndrome.[9,10] However, our observations indicate that the antiarrhythmic effects of Mg^{++} were indeed due to its pharmacological properties, because a similar magnitude of ARI shortening by Mg^{++} was observed before and during drug-induced autonomic block.

Distribution of Ventricular Repolarization

The Mg^{++}-induced changes in ventricular repolarization across the LV and RV walls were examined during ventricular pacing at 80 bpm. As observed by others in this model, ARI at the Mid sites was longer than at the Epi/Endo sites in both ventricles at baseline.[11,12] This was probably due to weaker net outward currents, particularly I_{ks}, and enhanced net inward currents, through late Na^+ current and Ca^{++} current, at the Mid sites.[13,14] Myocardium with M-cell characteristics was predominantly present in the LV, since ARI in the Mid layer was longer in the LV than in the RV.[11,12]

The antiarrhythmic effects of Mg^{++} in polymorphic ventricular tachyarrhythmia have been attributed to a decrease in inward Ca^{++} current via L-type Ca^{++} channels or to the stabilization of the membrane potential by facilitation of K^+ entry into the cells, from the enhancement of Na-K ATPase as a cofactor, or both.[4,15] Previous studies have reported little shortening of ventricular repolarization by Mg^{++},[16,17] although the results of our study are not concordant with these previous observations. One other study, however, found a significant shortening of ventricular repolarization by Mg^{++}.[18] These discordant observations may have at least 2 explanations. First, the antiarrhythmic effects of Mg^{++} have usually been studied in models of type 2 long QT syndrome, and the antiarrhythmic effects of Mg^{++} may vary among subtypes of long QT syndrome. Second, the magnitude of QT interval prolongation is greater in the anthopleurin-A model than in other experimental models or clinical observations. Therefore, a small decrease in net inward current by inhibition of Ca^{++} current by Mg^{++} would have a relatively large shortening effect on ventricular repolarization. This possibility might partially explain the greater ARI shortening by Mg^{++} in the Mid sites, which have the longest repolarization, than in the other ventricular layers, resulting in the homogenization of LV and RV transmural dispersion and decrease in interventricular difference of ARI.

Suppression of Ventricular Tachyarrhythmias

Triggered premature activity originating from the Purkinje network or subendocardium and heterogeneity of ventricular repolarization are two key factors in the initiation and perpetuation of polymorphic ventricular tachyarrhythmia

in the context of prolonged QT interval.[19,20] Before drug-induced autonomic block, polymorphic ventricular tachyarrhythmia developed during bradycardia in all experiments, in contrast to a single experiment during drug-induced autonomic block. Since drug-induced autonomic block caused little changes in the underlying dispersion of ventricular repolarization, the decrease in the incidence of premature events is the most likely explanation for the suppression of polymorphic ventricular tachyarrhythmia. The mechanism of triggered premature activity in long QT syndrome has been attributed to early afterdepolarizations.[20,21] Therefore, the suppression of triggered premature events by beta-adrenergic blockade (propranolol), which was a component of drug-induced autonomic block in this model, was not unexpected. However, beta-adrenergic blockade alone in patients suffering from type 3 long QT syndrome might be insufficient, because it slows the heart rate and increases the heterogeneous distribution of ventricular repolarization. Furthermore, it is usually difficult to inhibit all triggered premature activity by beta-adrenergic blockade.

On the other hand, the antiarrhythmic effects of Mg^{++} against polymorphic ventricular tachyarrhythmia were associated with both suppression of triggered premature activity and greater homogeneity of ventricular repolarization in this model.[3] However, the shortening of ventricular repolarization is associated with inhibition of triggered premature activity due to early after depolarizations. Therefore, the direct action of Mg^{++} on triggered premature activity was not fully examined in this study.

Characteristics of This Experimental Model

In this study, we created complete atrioventricular block to control the heart rate and to facilitate initiation of spontaneous ventricular tachyarrhythmias. Although we used back-up pacing at 100 bpm during the preparation of the experiment and a slower heart rate of 40 bpm was only applied during the study protocol, the slower heart rate associated with atrioventricular block has the potential to modify several potassium currents of the myocardium and may have affected the results of this study to some degree. Although several studies have previously reported that modulation of the myocardial potassium currents by a slower heart rate usually takes from a few days to weeks,[22,23] this model can not be considered as a pure model of type 3 long QT syndrome.

Clinical Implications

The suppression of triggered premature events by pharmacological interventions, including beta-adrenergic blockade, while effective, seems to be insufficient in the treatment of type 3 long QT syndrome patients.[24] However, it might be useful to limit the delivery of shocks in patients treated with implantable cardioverter defibrillators.

Study Limitations

A first limitation of our study was the limited number of needle electrodes and myocardial areas explored, which might have underestimated the heterogeneity of ventricular repolarization. The steepest repolarization gradients between the surface of the epicardium and the deep subepicardium are difficult to detect with unipolar electrograms that begin at a depth of 0.5 mm. The origin of triggered premature activity was not determined. However it is likely that the electrophysiological mechanisms of polymorphic ventricular tachyarrhythmia in this study were the same as those previously observed by detailed mapping in the same experimental model.[7,8] Second, bradycardia was applied for 60 sec, as the effects of intravenous Mg^{++} are relatively short-lived.[3] However, this brief observation period might have overestimated the antiarrhythmic effects of drug-induced autonomic block against polymorphic ventricular tachyarrhythmia. A longer period of bradycardia might have increased the likelihood of polymorphic ventricular tachyarrhythmia during drug-induced autonomic block. Third, we created atrioventricular block to control the heart rate, but no ion channel studies were performed. It was therefore unclear which ion channel was remodeled after the creation of atrioventricular block. Fourth, we did not measure the autonomic nerve discharges directly, making it unclear whether or not intravenous administration of Mg^{++} induced the modulation of autonomic nerve activity and resulting in conclusions based on the findings of pharmacological intervention to the autonomic nerve system. The role played by sympathetic versus parasympathetic nervous activity with respect to polymorphic ventricular tachyarrhythmia was not examined separately. Furthermore, the dose of propranolol in this study seems to be relatively small when compared with other experimental studies. However, because of the prolongation of the basic cycle length, suppression of triggered premature beats and mild decrease of systemic blood pressure, we think that the effect of the beta-blocker was obvious regarding the dose of this drug. Alteration of alpha-adrenergic activation may be related to the antiarrhythmic effect of Mg^{++} in this model. However, the role of alpha-adrenergic activation was not clarified because we did not use an alpha-adrenergic blocker in this study. Fifth, the animals were anesthetized with thiopental, which might antagonize the increase in late Na^{+} current by anthopleurin-A as well as synergize the effects of Mg^{++}. Intracellular Mg^{++} level is important in considering the therapeutic effect of Mg^{++} for polymorphic ventricular tachyarrhythmia, but this could not be measured in this study. Sixth, the cardiac loading condition must be altered secondary to mild depression of blood pressure by the administration of Mg^{++}, and this might affect to some degree the results of the antiarrhythmic effect of Mg^{++} in this model. Finally, there are several causes of QT interval prolongation. Therefore, the effects of Mg^{++} may be different among different etiologies of prolongation of repolarization.

CONCLUSIONS

The suppression of triggered premature ventricular events and homogenization of ventricular repolarization in this animal model of long QT syndrome was associated with the direct pharmacological properties of intravenous Mg^{++}. The suppression of triggered premature events without significant changes in the underlying dispersion of repolarization was partially effective in suppressing polymorphic ventricular tachyarrhythmia.

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Shortening of the ventricular fibrillatory intervals after administration of verapamil in a patient with Brugada syndrome and vasospastic angina

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Abstract A 43-year-old man presented with electrocardiographic findings consistent with Brugada syndrome. Though the baseline coronary angiogram was normal, intracoronary infusion of ergonovine maleate caused complete occlusion of the left anterior descending and a 99% occlusion of the proximal right coronary artery, each relieved by intracoronary isosorbide dinitrate. Double extrastimuli delivered at the right ventricular outflow tract induced ventricular fibrillation terminated by a 200-J shock. Verapamil, 10 mg IV, increased ST-segment elevation and programmed stimulation repeated after the drug induced ventricular fibrillation with shorter F-F intervals and lower amplitude signals, which was not terminated by 200 J and required an additional 360-J shock. Ca^{2+} antagonism may have been adverse in this patient with Brugada syndrome because the drug has the potential to increase the voltage gradient through the right ventricle and to slow intraventricular conduction at very fast heart rates.

Keywords: Brugada syndrome; Vasospastic angina; Calcium antagonism; Verapamil

Introduction

Brugada syndrome and vasospastic angina are separate cardiovascular disorders, which may occur concomitantly.[1,2] Although Ca^{2+} antagonists are the treatment of first choice for vasospastic angina, they can exacerbate the characteristic electrocardiographic abnormalities and ventricular fibrillation (VF) of Brugada syndrome. We report a patient with vasospastic angina and Brugada syndrome, in whom the administration of intravenous verapamil had adverse effects on the electrocardiographic manifestations and on the termination of VF.

Case report

A 43-year-old man was referred to our hospital for evaluation of an abnormal electrocardiogram (ECG) observed during a medical examination. He reported having suffered from atypical daytime chest oppression without syncope before this evaluation. He had no family history of sudden death or fainting. The resting ECG showed normal sinus rhythm and saddleback or, occasionally, coved-type ST-segment elevation in leads V_1 through V_2.[3] The results of hematologic and serologic examinations, chest radiograph, and echocardiogram were within normal limits. Late potential was positive in signal-averaged ECG. The diagnosis of Brugada syndrome was confirmed when 40 mg of pilsicainide, administered intravenously over 4 min, resulted in further ST-segment elevation and accentuation of the coved-type ST-segment abnormality (Fig. 1). The patient did not agree with the genetic analysis of Brugada syndrome.

The patient underwent cardiac catheterization to rule out vasospastic angina as the cause of his chest discomfort. The intracardiac pressures, left ventriculogram, and initial coronary angiography were normal. However, the injection of 50 μg of ergonovine maleate into the left coronary artery resulted in complete occlusion of the proximal left anterior descending artery, associated with further ST-segment elevation in leads V_1 through V_3 and development of chest oppression, both relieved within a few minutes by the immediate intracoronary injection of isosorbide dinitrate, after which, the patient's chest pain was relieved and the ST-

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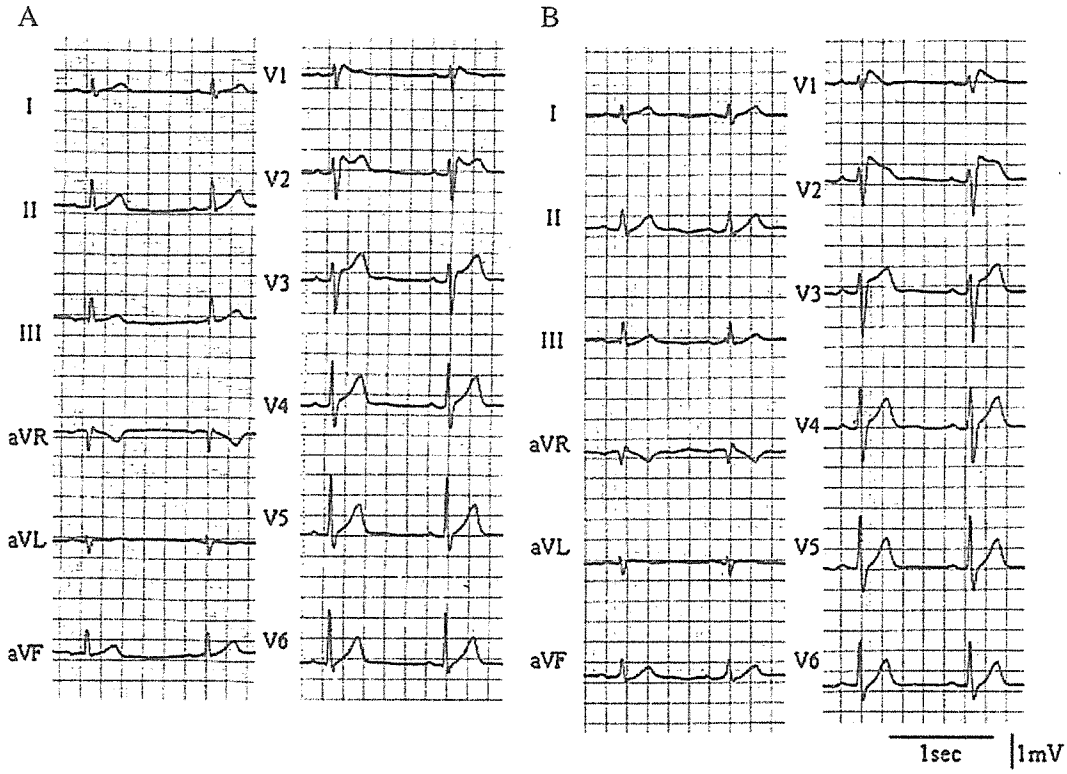

Fig. 1. Twelve-lead ECG before (panel A) and after (panel B) the administration of pilsicainide 40 mg over 4 minutes. Pilsicainide accentuated the ST-segment elevation in leads V_1 through V_3, and coved-type ST-segment elevation has become apparent.

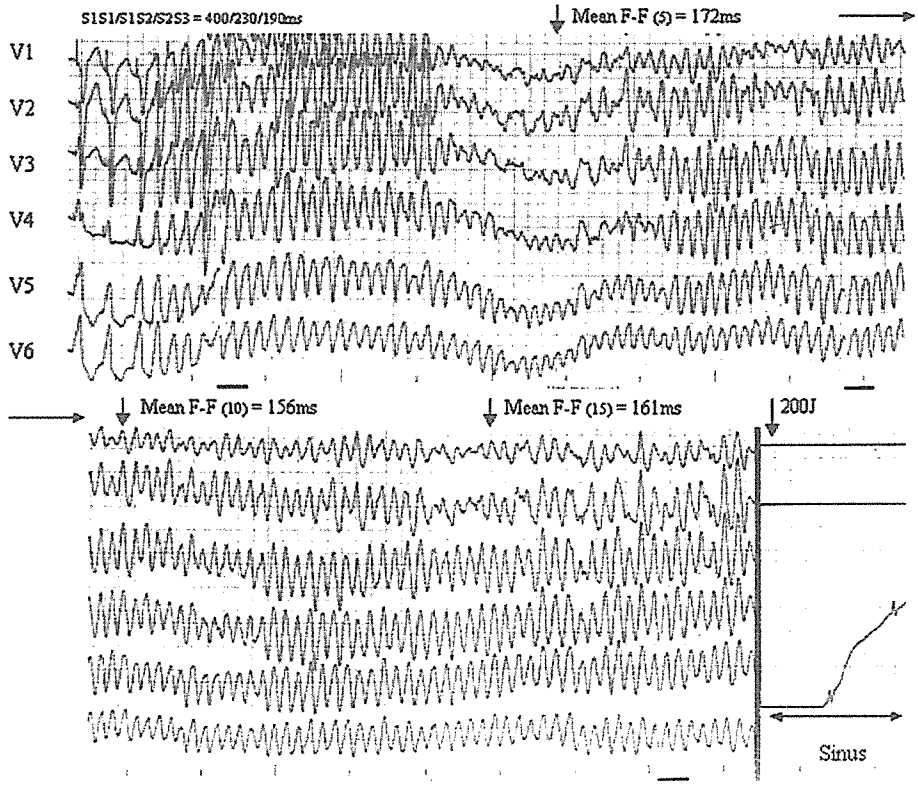

Fig. 2. Ventricular fibrillation induced by double ventricular extrastimuli delivered at the RVOT was terminated by a 200-J transthoracic DC shock 18.5 seconds after the onset of fibrillation. The numbers of QRS complex were counted over consecutive 5-second intervals, and the mean F-F interval was calculated as the numbers of QRS complex per 5 seconds. F-F(5) indicates mean F-F interval between onset of fibrillation and 5 seconds later; F-F(10), mean F-F interval between 5 and 10 seconds after the initiation of VF; F-F(15), the mean F-F interval between 10 and 15 seconds after the initiation of VF.

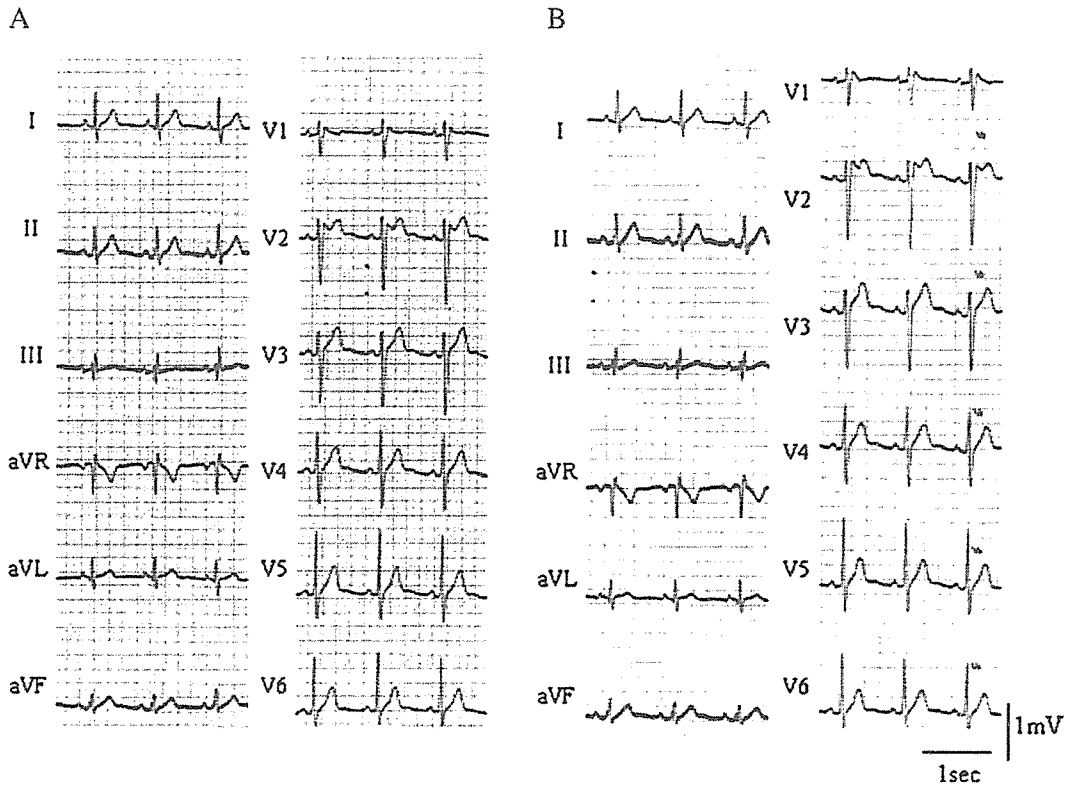

Fig. 3. Twelve-lead ECG before (panel A) and after (panel B) the administration of verapamil. Verapamil slightly increased the ST-segment elevation in leads V_1 and V_2.

segment elevation returned to baseline. Similarly, the injection of 50 μg of ergonovine maleate into the right coronary artery was followed by a 99% occlusion of its proximal segment, accentuation of ST-segment elevation in leads V_1 through V_3, and T-wave inversion in leads II, III, and aVF. After injection of isosorbide dinitrate into the right

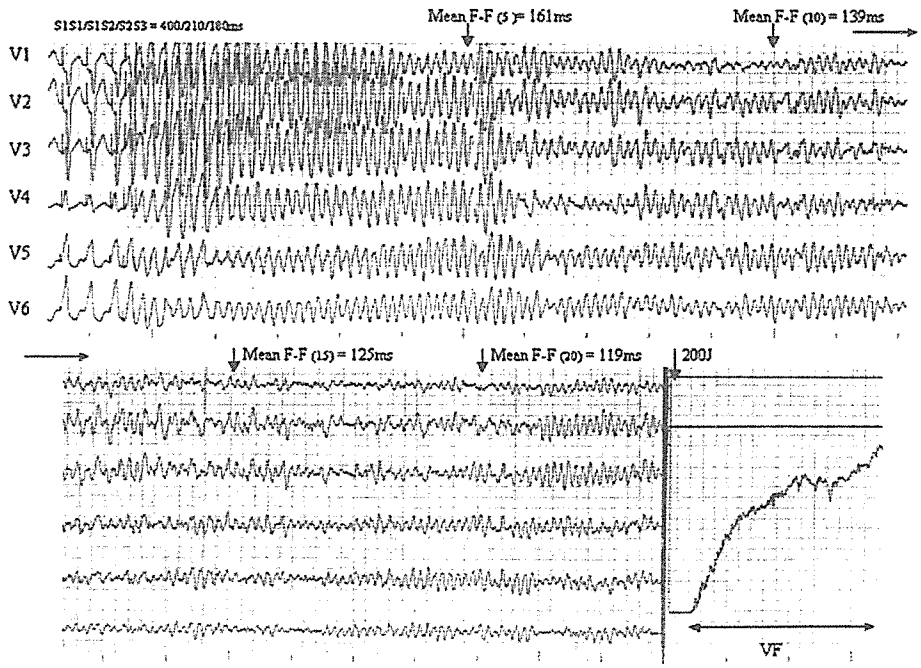

Fig. 4. Ventricular fibrillation induced by double ventricular extrastimuli delivered at the RVOT after the administration of verapamil. The mean F-F interval 10 seconds after the onset of the second VF episode is shorter than the corresponding mean interval during the first episode (Fig. 2). A 200-J transthoracic DC shock delivered 22.0 seconds after the initiation of VF failed to terminate the fine VF, and an additional 360-J shock was required to restore sinus rhythm. The mean F-F intervals were calculated as described in Fig. 2.

coronary artery, the vasospasm was relieved and the ECG returned to baseline.

The patient underwent programmed electrical stimulation, during which, double extrastimuli (S_1-S_1, S_1-S_2, and S_2-S_3 = 400, 230, and 190 milliseconds, respectively) delivered at the right ventricular (RV) outflow tract induced VF, successfully terminated 18.5 seconds later by a single 200-J, anteroposterior, transthoracic direct current (DC) shock (Fig. 2). Because we were planning to treat the patient's vasospasm with a Ca^{2+} antagonist, which has the potential to increase the risk of adverse cardiac effects in Brugada syndrome, programmed stimulation was repeated after verapamil, 10 mg, was administered intravenously. Before verapamil, the HV interval and conduction time interval between RV outflow tract (RVOT) and RV apex during pacing at a cycle length of 400 milliseconds measured 45 and 30 milliseconds, respectively, values that remained unchanged after administration of the drug. In addition, the patient's blood pressure remained stable (118/64 mm Hg before induction of the first VF episode, 124/66 mm Hg before verapamil, and 120/60 mm Hg after verapamil) throughout the electrophysiological study, and the RV effective refractory period, measured during pacing at a cycle length of 400 milliseconds, was 220 milliseconds before and 200 milliseconds after verapamil at the apex, and 190 milliseconds both before and after verapamil at the outflow tract. However, after the administration of verapamil, the ST-segment elevation in leads V_1 and V_2 increased by 0.1 to 0.2 mV (Fig. 3).

After the administration of verapamil, double ventricular extrastimuli delivered at similar coupling intervals (S_1-S_1, S_1-S_2, S_2-S_3 = 400, 210, and 180 milliseconds, respectively) at the RVOT reinduced VF, which was characterized by a shorter F-F interval and smaller signal amplitude than during the first episode (Fig. 4). A 200-J DC shock, delivered with the same patch electrode configuration 22.0 seconds after the induction of VF, was unsuccessful, and an additional 360-J shock was required to restore sinus rhythm. The implantation of a defibrillator was scheduled as a precautionary measure against life-threatening ventricular tachyarrhythmias.

Discussion

This patient presented with vasospasm of 2 major coronary arteries and Brugada syndrome. The prescription of a Ca^{2+} antagonist was strongly indicated for the prevention of adverse cardiac events because of acute myocardial ischemia. However, the inhibition of $I_{Ca^{2+}}$ by a Ca^{2+} antagonist may increase the voltage gradient between the RV endocardial and epicardial layers, exacerbate the Brugada-type abnormalities on surface ECG, and promote the development of VF.[4,5] Therefore, we examined the effects of the Ca^{2+} antagonist verapamil on the inducibility of VF by programmed ventricular stimulation. After the intravenous administration of verapamil, VF with shorter fibrillatory intervals and smaller signal amplitude was induced by the same stimulation techniques as before the drug was administered. Although the means to defibrillate were unchanged between the 2 episodes, the fine VF observed during the second episode was not terminated by a single 200-J shock and required a second stronger shock. The explanation for the development of fine VF is unclear and may be unrelated to the administration of verapamil. At the basic rhythm and during the pacing at 400 milliseconds, no significant differences were observed in the RV electrophysiological characteristics before and after the administration of verapamil. Previous reports showed that verapamil exerts little effects on the conduction properties or refractoriness of the normal ventricular myocardium during physiological heart rates,[6] but the electrophysiological properties of verapamil are use dependent, and in this patient, the intraventricular conduction delays that probably occurred during very rapid ventricular activation, as is the case during VF, may explain the further development of fine VF. We have recently reported that in Brugada syndrome, VF is usually characterized by shorter F-F intervals than in other patient populations.[7] Therefore, in patients with Brugada syndrome, the electrophysiological effects of Ca^{2+} antagonists may be more marked than in patients with other types of organic heart disease. Because we evaluated the conduction time between the RVOT and apex of the RV only at the pacing cycle of 400 milliseconds before and after use of verapamil in this patient, the intraventricular conduction delay that probably occurred during fine VF was not confirmed by electrophysiological measurement. Furthermore, in this patient, verapamil slightly increased the ST-segment elevation in leads V_1 and V_2. The transmural voltage gradient in the RVOT may, thus, have been increased,[4,5] and the resultant shorter action potential duration may have contributed to the development of fine VF. It seemed to be reasonable that the abbreviation of action potential duration especially in the epicardial layer of the RV was not represented as shorter effective refractory period in the RVOT because programmed electrical stimulation was applied from the endocardial site of the RV. Our observations in this patient were consistent with the results of a recent experimental study of Brugada syndrome,[5] in which verapamil augmented ST-segment elevation and facilitated initiation of VF. However, previous studies in isolated rabbit hearts reported the conversion of VF into monomorphic ventricular tachycardia in the presence of verapamil, despite the drug-induced shortening of the ventricular effective refractory period.[8] There may be differences in the effects conferred by Ca^{2+} antagonists to individuals with Brugada syndrome vs patients suffering from other types of cardiovascular disorders, though the precise reason for the discrepancy between previous observations and ours is unclear. Furthermore, different groups of Ca antagonists may differ in their electrophysiological properties, and they may not show such adverse effects in Brugada syndrome. There has been at least one case report using diltiazem successfully in a patient of Brugada syndrome with coronary artery spasm.[9]

Myocardial injury caused by the first defibrillation shock may have contributed to the subsequent induction of finer VF. However, this mechanism seems unlikely because his cardiovascular status was normal and his systemic blood pressure remained stable throughout the study. Furthermore,

the second VF induction was attempted more than 20 minutes after the first defibrillation shock, when the patient was hemodynamically stable. A slightly delayed delivery of the first shock (22.0 seconds after in the second induction vs 18.5 seconds after in the first induction) could have resulted in unsuccessful defibrillation of the second VF episode by the 200-J shock, though this seems unlikely. It is noteworthy that in the second VF episode, the evolution of the rhythm into fine VF with short F-F intervals became apparent within 10 seconds after the onset of VF (Fig. 3). Finally, it seems to be possible that the presence of a Brugada syndrome substrate sensitized this patient to the effect of ischemia. Further studies are required to clarify clinical implications of the coexistence of Brugada syndrome and vasospastic angina.

In conclusion, Ca^{2+} antagonists may cause adverse effects in patients with Brugada syndrome and vasospastic angina. Clinicians should therefore be aware of the possible development of fine VF or of an increase in the defibrillation energy requirements, or of both, after the administration of a Ca^{2+} antagonists in these patients.

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Over-expression of Kv1.5 in rat cardiomyocytes extremely shortens the duration of the action potential and causes rapid excitation

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Abstract

Background: Genetically abnormal action potential duration (APD) can be a cause of arrhythmias that include long and short QT interval syndrome.

Purpose: The aim of this study was to evaluate the arrhythmogenic effect of short QT syndrome induced by the over-expression of Kv1.5 in rat.

Methods: From Sprague–Dawley rats on fetal days 18–19, cardiomyocytes were excised and cultured with and without transfection with the Kv-1.5 gene using an adenovirus vector. The expression of Kv1.5 was proven by immunohistochemistry and Western blot analysis. In the culture dish and in the whole cells, the electrical activities were recorded using the whole-cell patch-clamp technique and the effects of 4-AP and verapamil were tested.

Results: After transfection with Kv1.5 for 12 h, immunohistochemical staining and Western blot analysis were positive for Kv1.5 while they were negative in the control transfected with only Lac-Z. In the culture dish, the myocytes showed spontaneous beating at 115 beats/min (bpm) just prior to the transfection with Kv1.5 and increased to 367 bpm at 24 h. The control myocytes showed stable beating rates during culturing. 4-AP at 200 μM slowed down the rate and verapamil abolished the beating. In the whole cells, the maximal resting membrane potential was slightly depolarized and APD was extremely abbreviated both at 50% and 90% of repolarization compared with those of the control. Rapid spontaneous activities were found in a single myocyte with Kv1.5 transfection and 4-AP slowed down the frequency of the activities with a reversal of the shortened APD.

Conclusion: The over-expression of Kv1.5 induced short APD and triggered activities in rat cardiomyocytes. This model can be used to study the arrhythmogenic substrate of short QT syndrome.

Keywords: Action potential duration; Kv1.5 channel; Gene expression; Short QT syndrome

The short QT interval has been established as a clinical entity of primary electrical heart disease and is associated with atrial fibrillation or sudden cardiac death [1,2]. Genetic screening showed missense mutations in cardiac potassium channels in the patients with the short QT syndrome: delayed rectifying K currents; IKs [3,4], IKr [5–7], and inwardly rectifying current IK1 [8]. A gain of function in these mutant channels is believed to abbreviate the action potential duration (APD) and results in the short QT interval [3,4,7,8].

As an arrhythmogenic basis, the transmural dispersion of repolarization is very important in the long QT syndrome, Brugada syndrome [9,10], and also in the short QT interval syndrome [11]. The increased heterogeneity of the repolarization is considered to precipitate phase-2 reentry and to lead to ventricular fibrillation [9,10].

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Kv1.5 is a gene encoding the ultrarapid delayed rectifier K^+ current (IKur) which is dominantly found in the atria but also in ventricles [12–16]. Kv1.5 is up-regulated by some factors including the thyroid hormone and is considered to provide the arrhythmogenic substrate for atrial fibrillation [14,15]. The overexpression of Kv1.5 shortens APD [15] and increases the peak currents of Ikur and shortens APD [16].

In the present study, we attempted to transfect the cultured fetal cardiomyocytes with Kv1.5 using an adenovirus [17] and observe rapid firing of myocytes with shortened APD. The spontaneous rapid electrical activities were observed even in a single cell. Then, 4-AP, a preferential blocker of IKur [12,16,18,19], was administered to reverse the effects induced by the over-expression of Kv1.5.

Methods

Primary culture and isolation of rat ventricular myocytes. A single litter of the Sprague–Dawley rats at fetal days 18–19 was used and under anesthesia, the hearts were quickly excised and put into ice-cold medium L-15. The ventricles were cut into 1-mm cubes and dissociated by stirring gently with 0.5 mg/ml collagenase solution for 15 min. Cells in the supernatant were removed and placed into ice-cold Dulbecco's modified essential medium (DMEM) with 0.5% fetal bovine serum (FBS). This extraction procedure was repeated 4–5 times.

The combined cell suspension was passed through 50-μm nylon mesh and centrifuged. The cell pellets, which consisted primarily of cardiomyocytes and nonmuscle cells, were resuspended in 10 ml DMEM with 10% FBS. The cell suspensions were incubated for 30 min at 37 °C in 100-mm dishes to remove fibroblasts. The cardiomyocyte-enriched suspension was removed from culture dishes and replated with DMEM containing 5% FBS and 0.1% penicillin–streptomycin. The cardiomyocytes were seeded at densities of 1.0×10^6 cells per 60-mm in a Corning tissue culture dish coated with gelatin. Each dispersion procedure yielded an average of 10 dishes from 10 fetal rat ventricles, which were divided for the experiments. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. In this experimental condition, the cultured cells began to beat spontaneously.

Adenovirus vector construction and transfection. The *Drosophila Shaker* Kv1.5 adenovirus vector was engineered and constructed following established protocol. Briefly, the full-length coding sequence of the Kv1.5 potassium channel was subcloned into an adenovirus shuttle vector provided by Dr. I. Sato (The Institute of Medical Science, The University of Tokyo, Japan). Ad-LacZ was used as the control vector. These viral vectors were propagated in human embryonic kidney 293 (HEK 293) cells.

Ventricular myocytes isolated and maintained in primary culture for 48 h were transfected (10–100 moi) with AdKv1.5 and Ad-LacZ. Six hundred microliters of culture medium plus the virus vector was added to each 60-mm dish. The dishes were incubated at 37 °C with gentle swirling every 15-min for 60 min, after which 4 ml of heart medium was added to each dish. At 6, 12, and 24 h after transfection, the adenovirus mediated expression of Kv1.5 was studied by Western blot analysis and immunochemistry.

Immunohistochemistry. Cells were fixed in 35-mm tissue culture dishes in 3.7% formaldehyde in phosphate-buffered saline (PBS: 137 mM NaCl/2.7 mM KCl/10 mM Na_2HPO_4/2 mM KH_2PO_4, pH 7.4) for 10 min at room temperature. Tissue culture dishes were then washed in PBS. Cells were blocked in 10% normal goat serum (NGS) (Jackson ImmunoResearch) for 2 h at 37 °C with the anti-Kv1.5 antibody (Alomone Labs Ltd.) at a 1:200 dilution in PBS/0.1% Triton X-100/1% NGS. After three 5-min washes in PBS, the dishes were incubated for 1 h at 37 °C with peroxidase-conjugated goat anti-rabbit antibody (Bio-Rad) at a 1:200 dilution in PBS/0.1% Triton X-100/1% NGS. After three 5-min washes in PBS, the peroxidase reaction was developed with Vectastain DAB (Vector Laboratories).

Western blot analysis. Cell lysates were prepared from virus-infected myocytes by using NP-40 lysis buffer [20 mM Hepes, pH 7.0/120 mM HCl/1 mM DTT/5 mM magnesium acetate/10% (vol/vol) glycerol/0.5% Nonidet P-40 containing proteinase inhibitors: 10 μg/ml each of leupeptin, aprotinin, and pepstatin, and 1 mg/ml Pefabloc] for 10 min on ice. The samples were centrifuged (13,800*g*) for 20 min (4 °C). The pellet was resuspended in 1% SDS sample buffer (60 mM Tris–HCl, pH 6.8/2% SDS/10% glycerol). Samples were boiled (5 min) and cell lysates were loaded onto SDS/8% polyacrylamide gel and subjected to electrophoresis. Protein was electrophoretically transferred (16 h, 50 V, 4 °C) to nitrocellulose membranes in Tris/glycine buffer [25 mM Tris–HCl, pH 8.3/150 mM glycine/10% (vol/vol) methanol]. The membranes were treated with blocking buffer consisting of 5% nonfat dried milk in Tris/saline buffer (0.9% NaCl/10 mM Tris–HCl, pH 7.5). For detection of the Kv1.5 protein, membranes were incubated with the anti-Kv1.5 antibody (Alomone Labs Ltd.) diluted 1:200 in blocking buffer for 1 h at room temperature. Primary antibodies were detected with goat anti-rabbit IgG conjugated with horseradish peroxidase (GIBCO/BRL Life Technologies) in blocking buffer. The antigen then was visualized with chemiluminescent substrates A and B (Kirkegaard and Perry Laboratories) and exposed to X-ray film.

Electrophysiological study. The whole-cell patch-clamp study was performed in a standardized manner [15,20,21]. A few drops of cell suspension were dispersed into a 0.5 ml superfusate bath on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan) and continuously superfused with standard Tyrode's solution at 36 °C. Myocytes were identified morphologically and clamped in the whole-cell configuration using an Axopatch 200B amplifier with a CV-203BU headstage and pClamp software (Axon Instruments, Foster City, CA). The resistance of the recording pipettes ranged between 3 and 5 MΩ when filled with pipette solution, which contained 5 mmol/l Na_2CrP, 0.5 mmol/l $MgCl_2$, 20 mmol/l TEACl, 10 mmol/l MgATP, 0.1 mmol/l Na_2GTP, 85 mmol/l aspartic acid, 10 mmol/l EGTA, 5.5 mmol/l glucose, and 10 mmol/l Hepes (pH 7.4 was adjusted with CsOH). Negative pressure was applied through the pipettes in the center of the myocytes, and the plasma membranes were perforated. The liquid junction potential (−10 mV) was corrected by voltage offset on the patch-clamp amplifier. Cell capacitance was measured using the internal circuit for capacitance-current compensation and cell capacitance and series resistance was compensated [15,20,21].

From the recording, the maximal membrane potential, the duration of the action potential (APD) at 50% and 90% of repolarization was determined in the KV1.5 transfected myocytes and the controls. The frequency of the spontaneous electrical activities was also determined in the two groups. The study was repeated after the addition of 4-AP to the medium.

In the culture dish, the frequency of spontaneous beating of the cardiac myocytes was obtained from the motion of the cell edge recorded on a videorecorder (SVT-S5100, SONY, Tokyo) and analyzed by a computer (POWER MAC 8600, Apple, USA) using a software for cell motion analysis (NIH image 1.62, Bethesda). The effect of 4-AP and verapamil was studied by their addition into the culture medium.

Statistical analysis. Values were expressed as means ± SD and compared between the Kv1.5 infected and the control myocytes by the unpaired *t*-test. A P value less than 0.05 was considered to be significant.

Results

Expression of potassium channel

The immunohistochemistry performed at 24 h after transfection showed positive staining for Kv1.5 while the control transfected with Lac Z was negative (Fig. 1A and B). Western blot analysis showed expression of Kv1.5 at

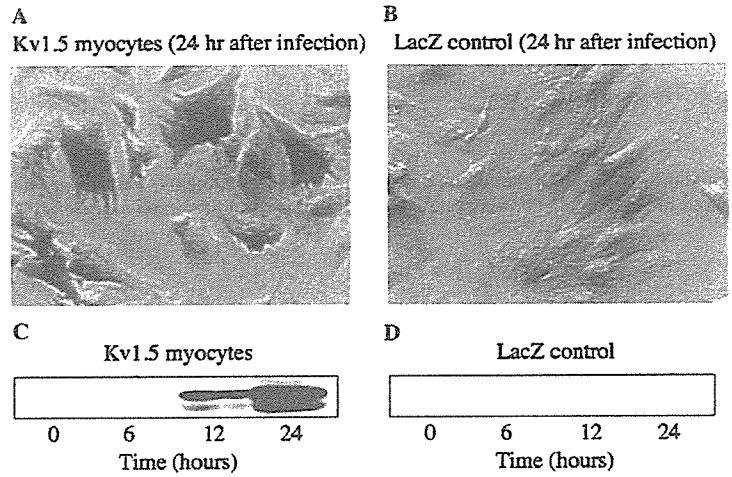

Fig. 1. Immunohistochemistry. (A) At 24 h after the transfection of Kv1.5, cardiomyocytes showed positive staining for Kv1.5 (A) but, such was not observed when Lac Z was transfected as the control (B). (B) Western blot shows expression of Kv1.5 at 12 h after transfection which increased further up to 24 h (C) while no expression was observed in the control (D).

12 h which increased towards 24 h of culture (Fig. 1C and D).

Spontaneous beatings of cultured myocytes

The myocytes showed spontaneous beating at 115 beats/min (bpm) just prior to the transfection with Kv1.5. At 6 and 12 h after transfection, the beating rate increased to 134 and 279 bpm, respectively, and at 24 h, disorganized beating at approximately 367 bpm was observed (Fig. 2A).

On the other hand, the control myocytes showed stable beating rates during culturing for 24 h (Fig. 2B).

At 24 h, when the myocytes were beating rapidly, 4-AP at 200 μM added to the medium slowed down the rate and the activities became more organized (Fig. 3A through C).

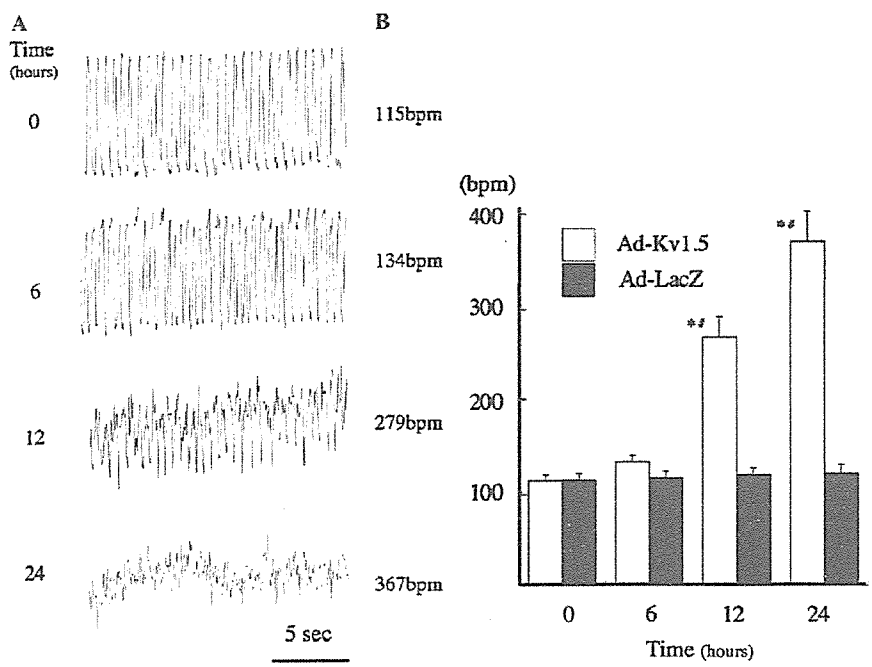

Fig. 2. Spontaneous beating of cardiomyocytes in the culture dish. At 0, transfection of Kv1.5 was attempted and myocytes showed regular spontaneous beating at 115 bpm. The beating rate significantly increased at 12 h and thereafter. At 24 h, the beating was very rapid and irregular (A.B). In the control culture, stable spontaneous beatings were observed and the rate did not accelerate with time (B). $^{*}P < 0.05$ versus 0 h. $^{\#}P < 0.05$ versus LacZ control.