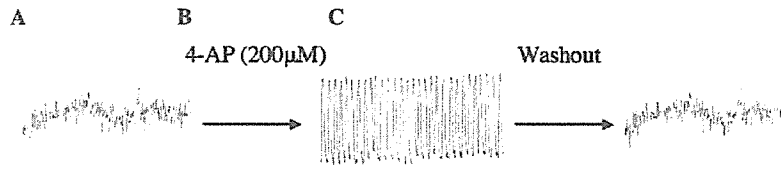

Fig. 3. The effect of 4-AP on the spontaneous beating in the culture dish. Rapid irregular beating (A) was changed into stable and slower rate by addition of 4-AP at 200 μM (B). After washout of the drug, the beating rate increased gradually and returned to the original level (C).

Table 1
The electrophysiological parameters of the myocytes after transfection

	Before 4-AP		After 4-AP	
	APD50 (ms)	APD90 (ms)	APD50 (ms)	APD90 (ms)
Control (Lac Z)	186 ± 32	296 ± 25	240 ± 9[a]	338 ± 9[a]
Kv1.5 transfection	35 ± 3*	77 ± 8*	148 ± 12*,[b]	205 ± 25*,[b]

[a] $P < 0.01$ versus before 4-AP.
[b] $P < 0.0001$.
* $P < 0.0001$ versus control.

When verapamil was added to the medium, the beating was completely abolished (not shown).

Electrophysiological parameters

The maximal resting membrane potential (Vm) was slightly depolarized in the mytocytes with Kv1.5 transfection compared with the control: 65.0 ± 7.1 mV versus 75.0 ± 5.0 mV ($P < 0.05$) and the cellular capacitance was larger but non-significant: 52.0 ± 5.7 pF and 44.3 ± 6.7 pF ($P > 0.1$), respectively.

As shown in Table 1 and in Fig. 4A and B, the myocytes transfected with Kv1.5 showed shorter APD both at 50% and 90% of repolarization compared with those of the control myocytes with Lac-Z transfection ($P < 0.05$). More rapid spontaneous activities were found in those with the Kv1.5 transfection (Fig. 4C and D). Addition of 4-AP slowed down the frequency of the activities and prolonged

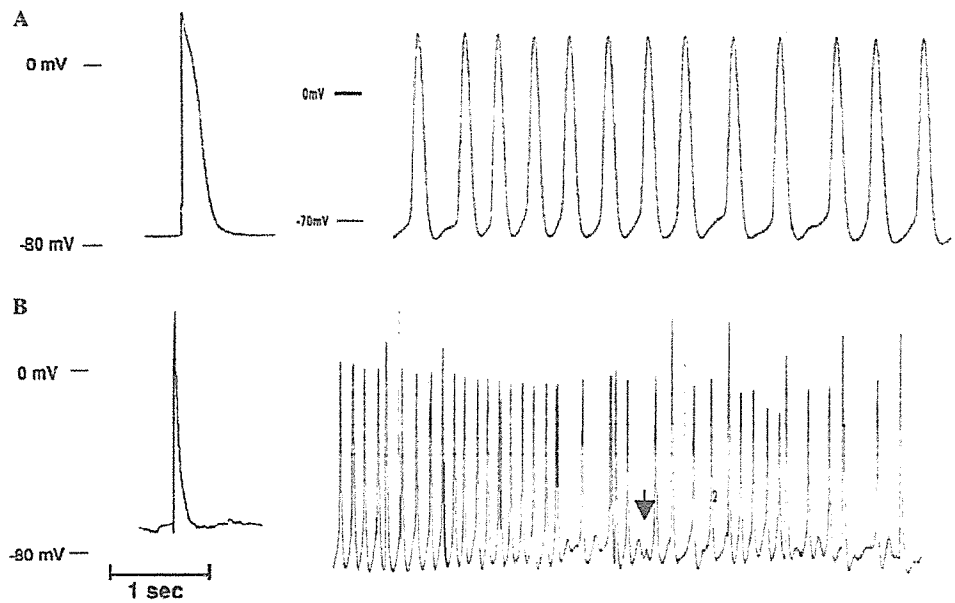

Fig. 4. The whole-cell patch-clam study of myocytes with and without Kv1.5 overexpression. In the control myocytes, APD was maintained and showed stable activities (A). The gradual diastolic depolarization might suggest automaticity. The myocyte with Kv1.5 transfection showed quite abbreviated APD and rapid and irregular activities were recorded (B). Delayed after-depolarizaion (DAD) was suggested as the cause of the activities as shown by arrow.

APD (Table 1). In the control myocytes, a single cell showed activities at a slower rate.

Discussion

Mutations of genes encoding cardiac ionic channels are the underlying cause of some fatal arrhythmias [3–8,22–25]. Quite often, mutation of genes of cardiac channels resulted in a loss of function [22–25] but, some mutations have been found to result in a gain of function [3,4,7,8]. In the latter case, abnormal potassium channels have led to shortening of APD: the short QT syndrome [3–8].

As the mechanisms of the arrhythmogenic substrate of the patients with short QT syndrome, increased transmural heterogeneity of repolarization was demonstrated and shown to precipitate Torsade de Pointes [11] as confirmed in long QT syndrome [9,24] and in Brugada sydrome [9].

By transfecting fetal cardiomyocytes with KV1.5 gene using an adenovirus as the vector, mRNA and channel protein were detected at 12 h and thereafter, and the over-expression of Kv1.5 was associated with a higher rate of spontaneous beatings and with extremely abbreviated APD. Rapid beatings in the cardiomyocytes in the dish can be explained by phase 2 reentry as a result of the heterogeneity of APD due to heterogeneous transfection and expression of the Kv1.5 gene [11]. However, such rapid excitation was observed even in a single myocyte overexpressed with the Kv1.5 gene and delayed afterdepolarization (DAD) was the likely mechanism (Fig. 4). 4-AP slowed the rate of the beating in the culture dish and the spontaneous activities of the myocytes with Kv1.5 over-expression. In the latter case, APD was prolonged by 4-AP (Table 1). Addition of a calcium channel blocker to the culture medium abolished electrical activity completely.

The control myocytes showed spontaneous activity with a slower rate than that found in myocytes with Kv1.5 over-expression and automaticity was the likely mechanism of the spontaneous activity (Fig. 4).

From the present study, it can be said that over-expression of Kv1.5 induces extreme abbreviation of APD in rat cardiomyocytes: a model of short QT syndrome and the whole-cell patch-clamp study suggested triggered activity as a result.

As a limitation, we did not elucidate the relationship between the extremely short APD (or short QT interval) and the spontaneous activities (DAD). The precise mechanism of rapid beatings of the myocytes with over-expression of Kv1.5 was not fully studied and we tested 4-AP and verapamil which abolished or slowed the spontaneous activities. We did specify the currents augmented by the over-expression of Kv1.5 but from previous studies, it is certain that Kv1.5 encodes Ikur.

Finally, if the adenovirus damaged myocytes and led to abnormal beatings or not was not determined but it was certain that extremely short APD was real and associated with abnormal excitation.

In summary, when fetal myocytes were cultured and Kv1.5 was over-expressed, extremely short APD and spontaneous rapid electrical activities were induced. This can be a model of the short QT syndrome and used to elucidate the arrhythmogenic substrate of the syndrome.

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Tumor necrosis factor-α inhibits the cardiac delayed rectifier K current via the asphingomyelin pathway

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Received 15 March 2006
Available online 29 March 2006

Abstract

Tumor necrosis factor-α (TNF-α) affects contractility and ionic currents in the heart. However, the electrophysiological effects, especially on delayed rectifier K currents (IK), have not yet been fully elucidated. We examined the effects of TNF-α on IK. Using a voltage-clamp method, IK was measured in guinea pig ventricular myocytes in the basal state and after pharmacological intervention. To specify the site of the action of TNF-α, the myocytes were incubated with pertussis toxin or *N*-oleoylethanolamine, a ceramidase inhibitor, and IK was measured. TNF-α suppressed IK when it was enhanced by isoproterenol, histamine or forskolin but not in the basal state or when IK was augmented by an internal application of cyclic AMP. Both pre-incubation with pertussis toxin and *N*-oleoylethanolamine abolished the inhibitory action of TNF-α on isoproterenol-augmented IK. TNF-α inhibits IK, mainly IKs, when it is augmented by PKA as a result of the generation of sphingosine.

Keywords: TNF-α; Delayed rectifier K current; c-AMP; PKA pathway; Sphingosine; Ceramide

Genes of tumor necrosis factor-α (TNF-α) are up-regulated in many myocardial diseases: ischemia [1], experimental and human myocarditis [2–4], dilated cardiomyopathy [5,6], and congestive heart failure [7–10], and can exert mechanical and electrophysiological effects on myocardial cells [11–14].

In the diseased heart, the PKA pathway is activated via enhanced adrenergic activity leading to the augmentation of the delayed rectifier potassium current (IK) as well as calcium currents [11,15]. However, the action of TNF-α on IK is unknown, so we studied the effect of TNF-α on IK in rat ventricular myocytes. We found that TNF-α inhibits IK but only when it was augmented by PKA activation and the effect was a result of the generation of sphingosine by TNF-α.

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doi:10.1016/j.bbrc.2006.03.115

Materials and methods

Cell preparation. Single ventricular myocytes were isolated from the left ventricle of adult guinea pigs weighing 250–400 g using an enzymatic dissociation procedure as reported previously [16]. Briefly, after deep anesthesia with pentobarbital sodium given intraperitoneally at 50 mg/kg, the chest was opened under artificial respiration, the aorta was cannulated with Langendorff's apparatus, and the heart was quickly excised. Using the retrograde perfusion, normal Tyrode's solution (36 °C) was applied for 5 min, followed by nominally Ca^{2+}-free Tyrode's solution until contraction ceased. Then, with Tyrode's solution supplemented with 0.4 mg/ml of collagenase type 1 (Sigma Co., St. Louis, MO), the heart was retrogradely perfused for 15–20 min. The composition of normal Tyrode's solution was NaCl 145, KCl 5.4, $CaCl_2$ 1.8, NaH_2PO_4 0.3, $MgCl_2$ 0.5, glucose 5.5, and Hepes 5 (pH adjusted to 7.4 with NaOH) (mmol/L).

Finally, the heart was perfused with KB medium at room temperature to rinse the collagenase off [17]. The composition of KB medium was L-glutamic acid 70, KCl 25, taurine 20, KH_2PO_4 10, $MgCl_2$ 3, EGTA 0.5, glucose 11, and Hepes 10 (pH adjusted to 7.3 with KOH) (mmol/L). The partially digested heart was gently minced with scissors in the KB solution and after filtration through 105-μm mesh, cells were stored at room temperature and used within 8 h of isolation.

Measurement of IK. The pipette solution contained potassium aspartate 110, KCl 20, $MgCl_2$ 7.0, $CaCl_2$ 0.69, K_2-ATP 5, Na_2-GTP 0.1, creatine phosphate-K_2 5, EGTA 5, and Hepes 5 (pH 7.4 with KOH) (mmol/L). According to the stabilizing constants proposed by Fabiato and Fabiato [18], with the correction of Tsien and Rink [19], the *p*Ca of the internal solution was calculated to be 8.0.

A few drops of cell suspension were dispersed into a small chamber superfused with Tyrode's medium on the stage of an inverted microscope (Olympus, Tokyo, Japan). A gigaohm seal was obtained in the center of the cells by applying negative pressure to the interior of the pipettes by gentle suction and the whole-cell currents were measured with low-resistance pipettes (2 MΩ) using an Axopatch 200B amplifier with a CV-203BU headstage and pClmap software (Axon Instruments, Foster City, CA).

In all experiments, the L-type calcium current (ICaL) was inhibited by 2 μmol/L of nisoldipine (Bayer Pharmaceutical Co., Osaka, Japan). The liquid junction potential was corrected by a voltage offset on the patch-clamp amplifier. Cell membrane capacitance was measured using the internal circuit for capacitance-current compensation. Series resistance was compensated for to minimize the duration of the capacitive surge.

After a depolarization pulse of 2 s, the voltage was clamped back to the holding potential (−40 mV) and IK steady-state currents were defined as the difference between the peak point of time-dependently activated component and the holding currents. All data were filtered at 1 kHz, digitized at 4 kHz using a Digidata 1200 (Axon Instruments, Foster City, CA), and stored on a custom-made computer (Intermedical, Nagoya, Japan). IK was measured in the basal state and after the addition of isoproterenol at 20 nmol/L and the pharmacological interventions were achieved as follows.

Measurements of IK in the altered PKA pathway. Rat TNF-α was dissolved in distilled water until use and the effect of TNF-α (Sigma Co.) was tested at 20 ng/ml before and after the addition of isoproterenol to the external medium. To examine the role of the intracellular signal transudation, we employed several pharmacological interventions.

Histamine is known to increase c-AMP and increase IK via the stimulatory G protein (Gs)/adenylate cyclase pathway [20]. Histamine (Sigma Co.) freshly made immediately before use was prepared to result in a 250 nmol/L and the effect of TNF-α was studied. Similarly, forskolin (Sigma Co.), a diterpene plant alkaloid known to increase intracellular c-AMP by directly stimulating adenylate cyclase [21], was dissolved in ethanol (10 mmol/L stock solution) and prepared at 500 nmol/L to augment IK and the effect of TNF-α was measured. Histamine and forskolin were administered to activate IK in a comparable magnitude as that of 20 nmol/L of isoproterenol. Then, c-AMP was administered intracellularly via direct dialysis of intrapipette c-AMP (1 μmol/L).

Since the intracellular c-AMP concentration is modulated by adenylate cyclase coupled with Gs and pertussis toxin (PTX)-sensitive G-protein (Gi), we examined the action of TNF-α on IK after the incubation of myocytes with PTX (Sigma Co.) at 5 μg/ml and at 36 °C for 120 min. After PTX treatments, we determined the effect of carbacol on IK [22].

Finally, we tested whether the ceramide–sphingosine pathway is involved concerning the action of TNF-α on IK. Sphingosine 1-phosphate, a product of the sphingomyelin pathway, has already been shown to modulate IKAch via PTX-sensitive G-proteins [23], therefore we incubated the myocytes with *N*-oleoylethanolamine (NOE) (Sigma Co.), a ceramidase inhibitor, and examined the effect of TNF-α on the

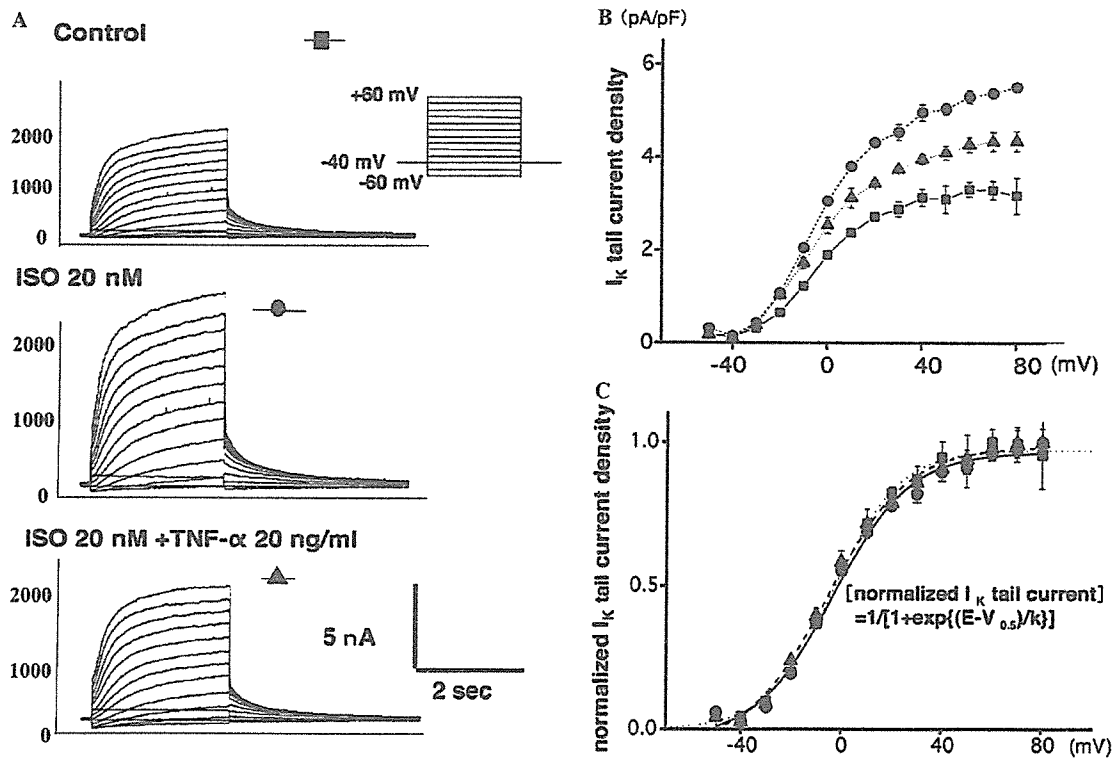

Fig. 1. The effects of TNF-α on current–voltage relationships for IK enhanced by isoproterenol. (A) Three sets of current traces at test potentials between −60 and +60 mV in 10-mV increments. (B) Tail current amplitudes plotted against test potentials. (C) Activation curves obtained by normalizing the IK tail current amplitudes. The solid curves are expressed as a function of membrane potential (E) as follows: normalized IK tail current = $1/\{1 + 1/\exp[(E - V_{0.5})/k]\}$, where k is the slope factor. Solid square indicates the control condition; solid circle, exposure to 20 nmol/L ISO; and solid triangle, after addition of 20 ng/ml TNF-α. Symbols and bars represent mean ± SE.

isoproterenol augmented IK. During the measurement of IK, the chamber perfusate was continuously drained by suction, and the complete exchange of the perfusate could be achieved in 2 min. The effect on membrane currents of solvents, DMSO (0.1%) and ethanol (<0.1%), was shown to be negligible.

Numerical data were presented as means ± SEM and they were compared by Student's *t* test. A *P* value of less than 0.05 was considered to be significant.

Results and discussion

Effect of TNF-α on IK

TNF-α caused no substantial effect on basal IK ($n = 4$) but when IK was increased to 179 ± 34% by isoproterenol at 20 nmol/L ($n = 20$), TNF-α reduced IK to 62 ± 10% (Figs. 1A and B). IK tail currents normalized by those measured after the application of a +80 mV potential test well fitted with the Boltzmann equation (Fig. 1C). Membrane potentials for $V_{0.5}$ were 8.4 ± 1.3 mV in the control condition, 9.6 ± 2.1 mV in the presence of isoproterenol, and 9.8 ± 1.2 mV after the addition of TNF-α. The threshold potential for IK activation was not altered by TNF-α. The dose–response curve of TNF-α on IK in the presence of 20 nmol/L isoproterenol ($n = 22$) was similar to the Hill equation (Fig. 2). TNF-α cancelled IK augmented by isoproterenol in a dose-dependent manner with IC_{50} at 11.6 ± 0.7 ng/ml and a Hill coefficient of 1.1 ± 0.1 and the inhibitory effect of TNF-α was not reversed after washout.

Histamine (250 nmol/L) enhanced IK tail current to 170 ± 69% as shown in Fig. 3A which was reversed by TNF-α (20 ng/ml) to 37 ± 13% ($n = 4$). Similarly, forskolin at 500 nmol/L augmented IK (175 ± 4.7%, $n = 5$) to a comparable degree as that induced by isoproterenol and TNF-α (20 ng/ml) again reversed IK to 58 ± 13% (Fig. 3B). However, direct dialysis of myocytes with intrapipette cAMP (1 μmol/L) caused an increase of IK but its reduction by TNF-α was negligible 8 ± 1% ($n = 5$) (Fig. 3C). These results suggested that TNF-α inhibits IK by reducing intracellular c-AMP.

IK is composed of two components: IKr and IKs [24]. Though IKr is partly sensitive to isoproterenol sensitive [25], we confirmed the same inhibitory property on IK after

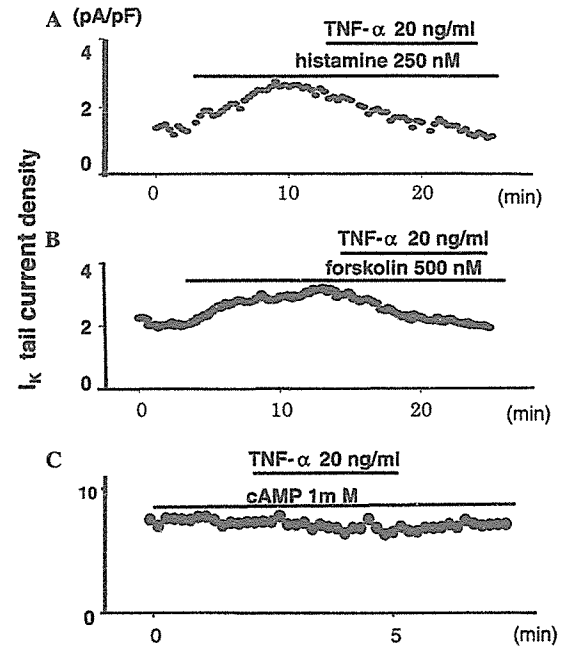

Fig. 3. The inhibitory effect of TNF-α on other types of PKA-enhanced IK. (A–C) Three time courses showing TNF-α (20 ng/ml) actions on the tail current of IK potentiated by histamine (250 nM/L), forskolin (500 nM/L), and intrapipette cAMP (1 mM/L).

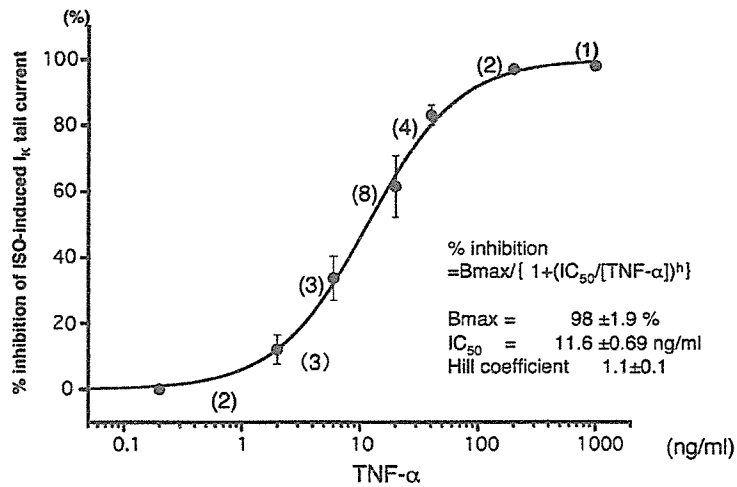

Fig. 2. The effects of TNF-α concentration–inhibition relationships for IK enhanced by isoproterenol. TNF-α dependent inhibitions accessed by normalizing amplitudes of IK tails after +40-mV test potential in the presence of various concentrations of TNF-α by that measured in the absence of the peptide and plotted as a function of the TNF-α concentration. Symbols represent the mean percent inhibition; the smooth line, the best fit to the Hill equation: % inhibition = $B_{max}/\{1 + (IC_{50}/[\text{TNF-}\alpha])^h\}$, where B_{max} indicates the maximal inhibition, and h, the Hill coefficient. Vertical bars represent SE. Numbers in parentheses indicate the number of experiments.

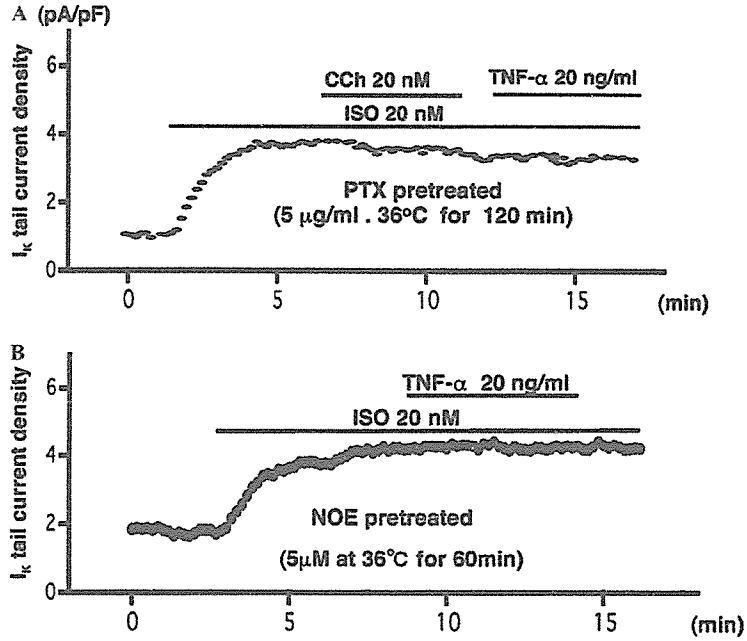

Fig. 4. The inhibitory effect of TNF-α is mediated by PTX-sensitive G-proteins and *N*-oleoylethanolamine. (A) Preincubation with PTX (5 μg/ml at 36 °C > 60 min) abolished the inhibitory effect of both CCh (20 nM) and TNF-α (20 ng/ml). (B) Preincubation with ceramidase inhibitor, *N*-oleoylethanolamine, also abolished the inhibitory effect of TNF-α (20 ng/ml).

the use of E-4031: an IKr inhibitor (up to 2 μmol/L) prior to the present study and the inhibitory effect of TNF-α on the isoproterenol activated IK would represent mainly that of IKs. TNF-α has no effect on basal IKs but reduced it when it is augmented by isoproterenol, histamine, or forskolin.

Effects of PTX and the ceramide–sphingosine phosphate pathway

Carbachol reduced intracellular c-AMP by stimulating the PTX-sensitive G-protein (Gi) and inhibited the isoproterenol-augmented IK but when the myocytes were preincubated with PTX, such action of carbachol was abolished and the addition of TNF-α showed no effect (Fig. 4A). Furthermore, when the myocytes were pre-incubated with *N*-oleoylethanolamine (at 5 μM), a ceramidase inhibitor, the inhibitory effect on IK by TNF-α was also abolished to 10 ± 4% (Fig. 4B).

Sphingosine-1-phosphate is considered to be generated from the ceramide-sphingomyelin by TNF-α and to activate Gi leading to a fall of c-AMP [26,27]. The fall of c-AMP must result in an immediate effect on contractile dysfunction [26,28–30] as well as on ionic currents [31–33] including IKs as shown in the present study.

In summary, TNF-α was shown to reverse IK when it is augmented by the PKA pathway. A fall of c-AMP would be involved as the mechanism of the reversal of IK by TNF-α since TNF-α induces a generation of sphingosine-1-phosphate from ceramide-sphingomyelin leading to the activation of Gi. However, because of diverse actions of TNF-α on several ionic currents, the net electrophysiological effects of TNF-α and their roles need to be further studied.

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Distinct U Wave Changes in Patients With Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

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SUMMARY

Although catecholaminergic polymorphic ventricular tachycardia (CPVT) is associated with fatal ventricular arrhythmias and sudden death, the ECG findings are not fully understood. In this paper, we report on alterations in the U-wave.

Seven patients from 6 families with CPVT in which bidirectional tachycardia and polymorphic VT were induced by exercise or isoproterenol infusion visited our hospitals. VT was not inducible by programmed electrical stimulation. A novel gene mutation of the ryanodine receptor 2 (RyR2) was confirmed in 2 families.

In one of these patients, U-wave alternans was observed following ventricular pacing at 160 beats/min. In the other patient, U-wave alternans was observed during the recovery phase after the exercise stress test, which was terminated because of polymorphic VT. In both cases, leads V_3-V_5 were the leads showing alternans most clearly. In the third patient, a negative U-wave became positive following a pause from sinus arrest and a change in T-wave was also noted.

Since such findings were not found in the other subjects who underwent electrophysiologic study, isoproterenol infusion or exercise stress testing, the phenomenon seems to be relevant to the underlying pathogenesis of CPVT. The genesis and significance of U-wave alteration need to be determined. (Int Heart J 2006; 47: 381-389)

Key words: Bidirectional ventricular tachycardia, Mutation, Ryanodine receptor

THE U-wave is normally recognized as a low amplitude wave following a T-wave, however, its genesis or origin remains controversial.[1-4] There are clinical settings in which abnormal U-waves can be observed: prominent U-waves in hypokalemia,[5] inverted U-waves in myocardial ischemia,[6-8] ventricular hypertrophy or dilatation,[9,10] or alteration in the amplitude in ischemic heart disease or in idiopathic ventricular tachycardia.[11]

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Received for publication November 28, 2005.

Revised and accepted February 3, 2006.

Recently, we experienced 7 patients with catecholaminergic polymorphic ventricular tachycardia (CPVT), which is one of the primary electrical diseases of the heart and associated with fatal ventricular arrhythmias.[12,13] Gene mutation in the cardiac ryanodine receptor (RyR2) has been confirmed in 50% of patients.[14-17] The mechanism of ventricular tachyarrhythmias in CPVT is considered to be a triggered activity due to delayed afterdepolarization (DAD).[18-21] DAD is known to be augmented if intracellular calcium overload occurs in the presence of an abnormal ryanodine receptor.[14,19-21]

Altered calcium cycling is now believed to result in alteration of repolarization and T-wave alternans and T-wave alternans is established as a strong predictor of arrhythmogenic risk,[22-24] but such an ECG marker of repolarization has not yet been evaluated in CPVT in which abnormal calcium cycling is highly possible.[17-21,25]

We observed macroscopic alteration in the U-wave: alternans in 2 patients and a change in the polarity after a pause among 7 patients with CPVT.

METHODS

Patients: Seven patients were referred to Niigata University Hospital and Okayama University Hospital for further evaluation of ventricular tachyarrhythmias. All patients had polymorphic ventricular tachycardia and bidirectional ventricular tachycardia with or without ventricular fibrillation. All of these arrhythmias were induced by exercise or by infusion of isoproterenol (Table). Routine examinations excluded structural heart diseases or ischemic heart disease. The ventricular tachycardias were not induced by programmed electrical stimulation.[26] Of these, 2 *de novo* mutations were confirmed in 3 patients from 2 families as reported elsewhere.[15,16]

Table. Clinical and ECG Profiles of Patients With CPVT

Case	Age/Sex	Syncope	HR (bpm)	QT (msec)	U (mV)	Arrhythmias documented
1	20/M	+	46	440	0.1	BVT/PVT*
2	23/F	-	60	420	< 0.1	PVT*
3	17/M	-	43	450	0.2	BVT/PVT*
4	34/F	-	50	400	0.2	PVT*
5	23/F	+	55	440	< 0.1	PVT**
6	57/F	+	53	420	0.3	PVT**
7	30/M	+	56	360	0.5	PVT**

BVT indicates bidirectional ventricular tachycardia and PVT, polymorphic ventricular tachycardia.
*:arrhythmias induced by isoproterenol infusion. **: arrhythmias induced in exercise test on treadmill or two-step test. Case 7 is the son of case 6.

ECG findings: The ECGs of 7 patients had normal PR and QT intervals as well as QRS complexes but they showed a relatively slow heart rate (43-60 beats/min.) The U-wave was within the normal range in all patients (Table).

The first patient was a 20-year-old male (case 1) and polymorphic ventricular tachycardia and ventricular fibrillation were induced on exercise at another hospital and DC shock was used to restore sinus rhythm. He visited Niigata University Hospital for further evaluation.

His resting ECG was normal (Figure 1A) and other examinations resulted in normal findings. No ventricular arrhythmia was induced in an electrophysiologic study by programmed ventricular stimulation, but infusion of isoproterenol induced frequent premature ventricular beats and bidirectional ventricular tachycardia as shown in Figures 1B and 1C.

The ECG was normal and showed a normal U-wave without alternation in the control state (Figure 2A). In an electrophysiologic study, rapid ventricular

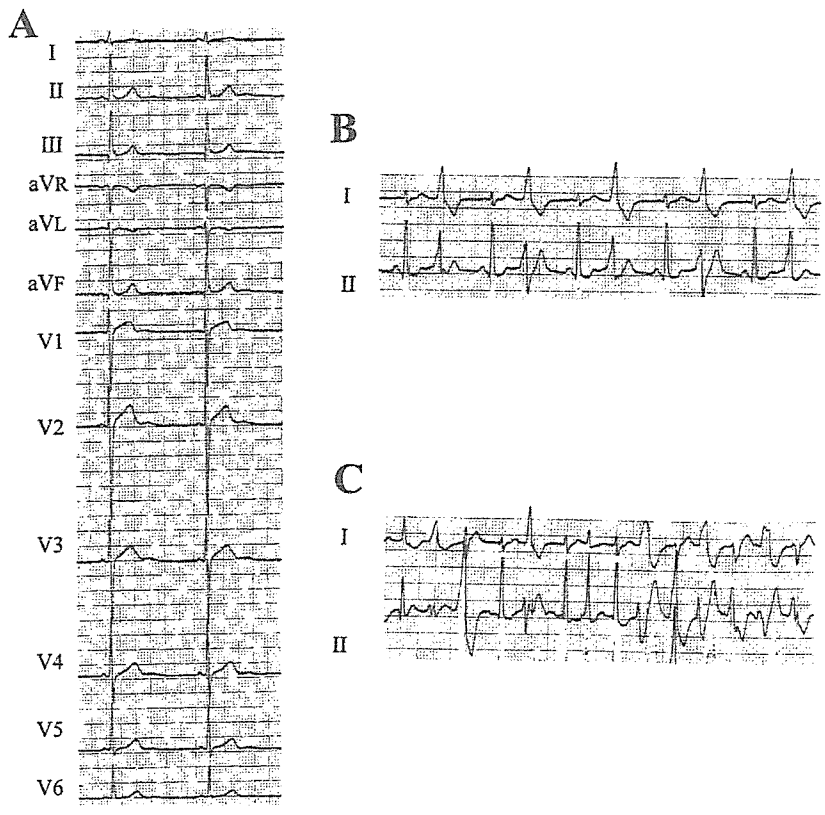

Figure 1. ECG of a patient with catecholaminergic polymorphic ventricular tachycardia.
The patient was case 1. **A:** 12 lead ECG shows entirely normal tracing. **B:** Infusion of isoproterenol in an electrophysiologic study induced a ventricular premature beat, which was followed by poly or bidirectional ventricular tachycardia as shown in **C**.

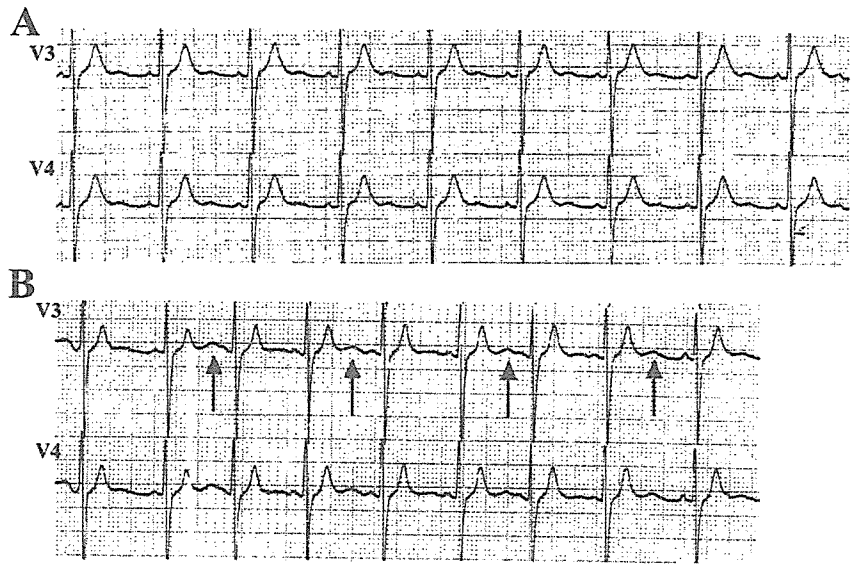

Figure 2. U-Wave Alternans.
Same patient as in Figure 1. **A:** Before the ventricular pacing, a small but stable U wave can be seen. Rapid ventricular pacing was performed for 10 seconds up to 210 beats/min to induce VT. **B:** After ventricular pacing at 160 beats/min, U-wave alternans can be clearly seen in V_3 and V_5. The RR interval showed variations but the alternans was not related to the varying preceding RR interval.

pacing was attempted at progressively higher rates and immediately after pacing at 160 beats/min for 10 seconds, an alternating U-wave was found in the precordial leads (Figure 2B). The RR interval showed a slight variation but the amplitude of the U-wave was not closely related to the variation of the RR interval. After rapid pacing at lower or higher rates, such alternation was not evident. A novel mutation was confirmed in the ryanodine receptor as reported elsewhere.[15]

The second patient was a 23-year-old female (case 2) and her resting ECG was noncontributory: sinus rhythm at 60 beats/min with normal PR and QT and a normal U-wave. She was referred to Okayama University Hospital. During two-step exercise testing, a polymorphic ventricular tachycardia was induced (Figure 3A) and exercise was stopped. Five minutes after the cessation of exercise, U-wave alternans was observed in the precordial leads, V_3-V_6 (Figure 3B). Though the RR interval varied a little, the amplitude of the U-wave was not related to the varying preceding RR interval. The phenomenon was transient and an ECG 8 minutes after exercise showed no alternans of the U-wave.

The third patient was a 57-year-old female (case 6 and the mother of case 7). She developed palpitations and syncope on exertion and Holter ECG showed frequent polymorphic ventricular tachycardia (Figure 4A). There was a history of sudden cardiac death in the family and a novel mutation was found in the ryano-

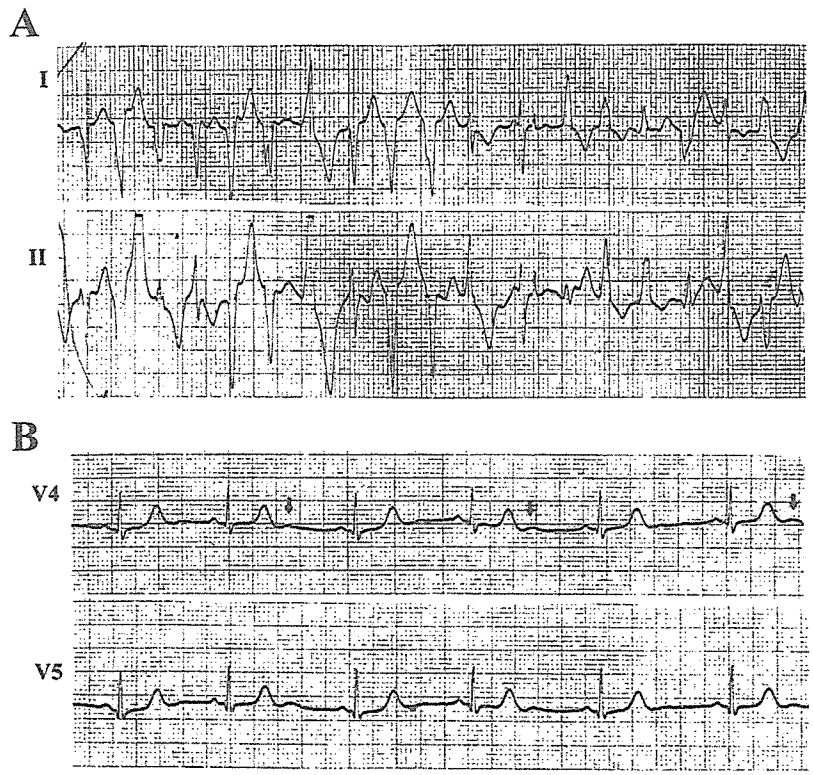

Figure 3. ECG of a patient with catecholaminergic polymorphic ventricular tachycardia.
The patient was case 2 and polymorphic ventricular tachycardia was easily induced by two-step exercise testing (**A**). It disappeared soon after cessation of the exercise and at 5 minutes into the recovery phase, U-wave alternans was observed in the precordial leads (**B**). They were most clearly in V_3-V_5.

dine receptor.[13] She was treated with beta-blockers which suppressed exercise induced ventricular arrhythmia, but only partially, and she was referred to Niigata University Hospital for further evaluation. Routine examinations were normal and structural heart disease was not found. The coronary angiography performed at another hospital was normal.

Her ECG showed normal tracing except for the negative U-wave in precordial leads V_4 to V_6. An intermittent pause during continuous tracing was due to sinus arrest or arrhythmia (Figure 4B). The beat following a pause showed a positive U-wave which returned as a negative one within 5-6 beats. This pause dependent change in the polarity of the U-wave was reproducible and a concomitant change in the T-wave was observed.

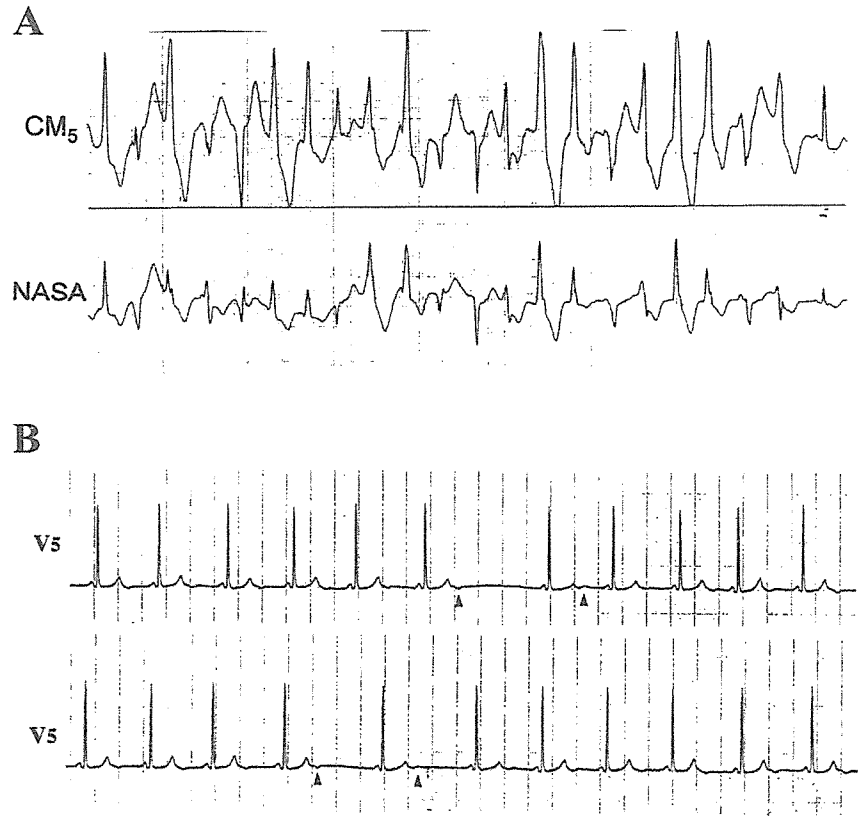

Figure 4. Pause-dependent change in U-Wave polarity.
The patient was a female (case 6). **A:** Holter ECG showed polymorphic ventricular tachycardia on exercise. She had been treated with a beta-blocker and then visited our hospital. Her ECG showed intermittent prolongation of the RR interval because of sinus arrest or sinus arrhythmia (**B**). Negative U-wave was clearly seen in the precordial leads but immediately after a pause, it became positive and returned to being negative in 5-6 beats (upper). Following sinus arrhythmia, it became less negative (lower). A gradual change in the T-wave was also noted.
CM5/NASA: Standard leads used in Holter ECG.

DISCUSSION

CPVT is a primary electrical disease of the heart and is associated with fatal ventricular arrhythmias.[12-15] The ventricular arrhythmias are precipitated by emotional and physical stress and recent genetic studies have shown a mutation in RyR2.[14-16,18]

The mutation of RyR2 in CPVT has been shown to increase the probability of the Ca-channel of sarcoplasmic reticulum opening, leading to intracellular Ca-overloading,[18-21] and the ventricular arrhythmias found in CPVT are believed to be due to a triggered activity.[17,18] However, there is no marker in the surface ECG

or in an electrophysiologic study predicting the arrhythmogenic risk in CPVT.

All 7 patients studied at the 2 institutions (Niigata University Hospital and Okayama University Hospital) could be diagnosed as CPVT. Of note, we found unexpected changes in the U-wave in 3 of the 7 patients: U-wave alternans in 2 patients and a change in the polarity of the inverted U-wave after a pause in 1 patient. The pause-dependent change in U-wave polarity was associated with a change in the T-wave.

Changes in the amplitude of the U-wave might be observed in some patients with ischemic heart disease during exercise stress testing[6,8] or following a pause after premature ventricular contraction in patients with idiopathic ventricular tachycardia[11] or long QT syndrome,[27] but alternans of the U-wave has not been reported. Similarly, though an inverted U-wave is considered as a hallmark for the presence of myocardial ischemia or hypertrophy,[6-10] a prompt change after a pause has not been previously reported as found in the present study. The mechanism and the significance of such peculiar changes in the U-wave need to be determined.

Macroscopic T-wave alternans is often observed in heart failure patients[28] or in patients with long QT syndrome[27] and is associated with a poor prognosis and can be a marker of arrhythmogenic risk. Furthermore, microvoltage T-wave alternans, which is a heart rate-dependent measure of repolarization,[22] has been shown to be a strong predictor of spontaneous ventricular arrhythmias or death.[22-24]

T-wave alternans has been shown to result from alteration of cardiac repolarization at the cellular level, and the heart rate at which myocytes exceed the capacity to cycle intracellular Ca^{2+} is crucial for T-wave alternans to develop.[29,30] In a single cell,[25] a tissue sample,[31] and in the intact heart,[32] T-wave alternans has now been shown to be closely associated with intercellular Ca cycling rather than action potential duration restitution.

Since the release of calcium from sarcoplasmic reticulum has been shown to promote both intercellular Ca^{2+} alternans and action potential duration alternans,[25] T-wave alternans might be expected to occur very often in CPVT, however, this has not yet been proven. The relation between the abnormal Ca^{2+} cycling and U-wave alternans needs to be determined in the future.

In summary, we have presented 3 patients with CPVT who showed U-wave alternans or a sudden change in the U-wave polarity after a pause. Altered intracellular calcium cycling in CPVT may be related to such peculiar ECG findings, however, its pathogenesis or relation to arrhythmogenicity needs to be studied further.

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Elimination of late potentials by quinidine in a patient with Brugada syndrome

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Received 23 March 2005; revised 8 July 2005; accepted 11 July 2005

Abstract The beneficial effects of quinidine on ST-segment elevation, inducible ventricular tachyarrhythmias, and episodes of ventricular tachyarrhythmia have been reported in Brugada syndrome. This is the first report describing quinidine-induced elimination of the late potential, which is considered one of the parameters for an arrhythmic event, in a patient with Brugada syndrome.

Keywords: Brugada syndrome; Quinidine; Late potential; Signal-averaged electrocardiogram

1. Introduction

Brugada syndrome is a distinct clinical syndrome associated with syncopal episodes and sudden cardiac death in patients with apparently normal hearts [1]. The syndrome is characterized by a right bundle-branch block pattern and ST-segment elevation in right precordial leads V_1 through V_3. Implantable cardioverter/defibrillator (ICD) is the only reliable treatment to prevent sudden death in Brugada syndrome [2]. Quinidine, a class IA agent with several ion current–blocking properties, including transient outward potassium current (I_{to}), is a candidate for pharmacologic treatment, and the beneficial effects on ST-segment elevation, inducible ventricular tachyarrhythmias, and ventricular tachyarrhythmia episodes have been reported [3-5]. This case report describes the elimination of late potentials in signal-averaged electrocardiogram (ECG) that were eliminated by quinidine in a patient with Brugada syndrome.

2. Case report

A 56-year-old man was admitted to our hospital for further evaluation of Brugada syndrome. He had experienced syncope 2 times during the last 5 years. He had a 15-year history of hypertension treated with diuretics and there was no family history of sudden death. Physical examination and routine laboratory tests were unremarkable. His ECG showed saddle back–type (type II) ST-segment elevation in lead V_2 [6]. Intravenous administration of pilsicainide (0.8 mg/kg) exaggerated the ST-segment elevation by 4 mm (0.4 mV) and changed the configuration of ST-segment into the coved type (type I). Consequently, Brugada syndrome was diagnosed in this patient. Right heart catheterization, left ventriculography, and coronary angiography were normal. Programmed right ventricular electrical stimulation induced ventricular fibrillation repetitively. He underwent implantation of ICD after granting informed consent.

After the ICD implantation, quinidine sulfate was administered at the standard dose of 200 mg 3 times a day. The blood concentration of quinidine was 2.0 μg/mL at the time 12-lead ECG and signal-averaged ECG were recorded to evaluate the drug effect. Quinidine did not change the

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doi:10.1016/j.jelectrocard.2005.07.009

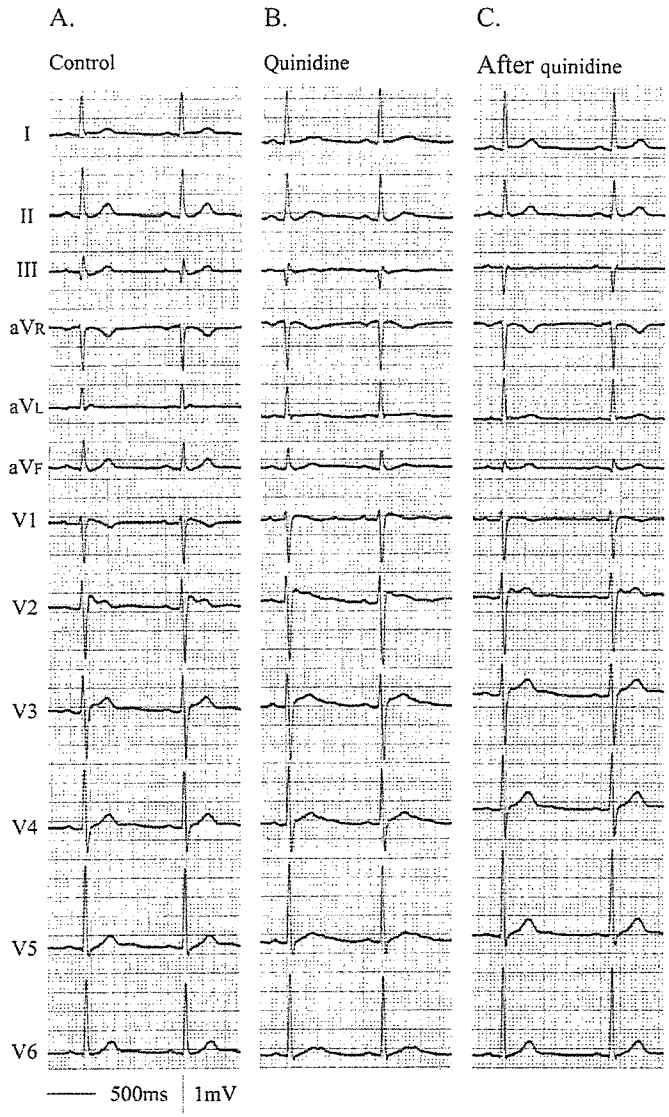


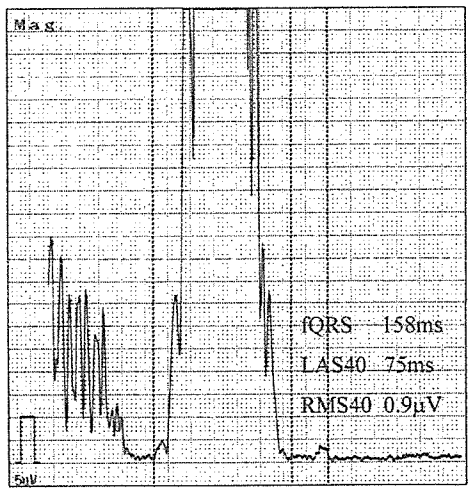
Fig. 1. Twelve-lead ECGs before (A), during administration of quinidine (B), and after discontinuation of quinidine (C). The ST-segment elevation in the right precordial leads did not change significantly.

magnitude of J-wave or ST-segment configuration markedly, whereas it prolonged the QT interval from 360 to 420 milliseconds and the corrected QT interval by Bazett's formula from 356 to 426 $ms^{1/2}$ (Fig. 1). The dispersion of QT interval that was measured manually as maximal QT interval difference in the 12-lead ECG was identical before and during the quinidine administration (80 milliseconds for each). According to the following criteria for late potentials, the root-mean-square voltage of the terminal 40 milliseconds in the QRS complexes (RMS40) less than 20 μV and the duration of low-amplitude signals less than 40 μV of the QRS complexes (LAS40) more than 38 milliseconds, late potentials were positive in the signal-averaged ECG before the administration of quinidine, as shown in Fig. 2 [7]. Late potentials became negative during the quinidine administration as the RMS40 increased and the LAS40 was shortened (Fig. 2B). The duration of the major component of the QRS complexes, which is represented by the difference between the total filtered QRS duration and LAS40 was similar before, during, and after the administration of quinidine. After discontinuation of quinidine due to the patient's desire to avoid long-term administration, the late potential reappeared (Fig. 2C).

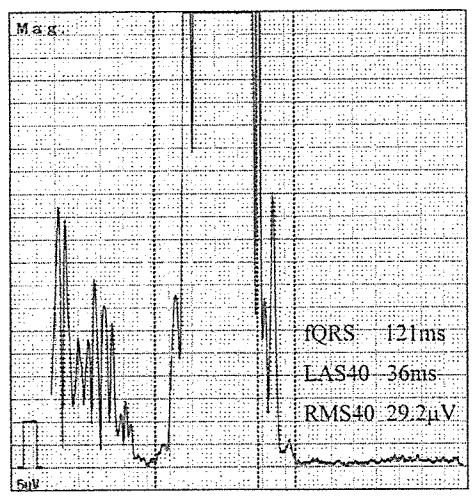
3. Discussion

This is the first report of the elimination of late potentials by quinidine in Brugada syndrome to the best of our knowledge.

A.



B.



C.

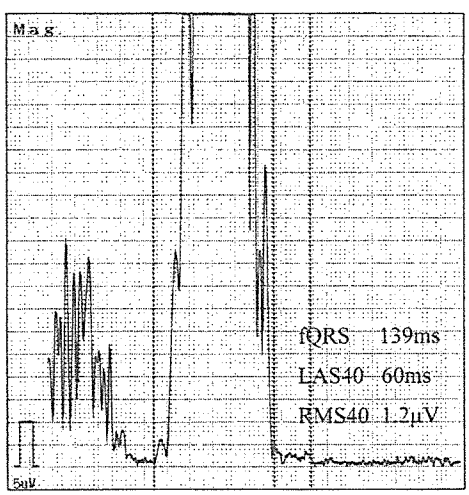


Fig. 2. Signal-averaged ECGs before (A), during administration of quinidine (B), and after discontinuation of quinidine (C). Late potentials disappeared during quinidine administration and reappeared after the discontinuation. fQRS indicates filtered QRS duration.

Loss of the action potential dome in the right ventricular epicardium is thought to account for the ST-segment elevation and the development of ventricular tachyarrhythmia in Brugada syndrome [3,8]. Therefore, pharmacologic treatment, which restores the abnormal action potential dome, should prevent ventricular fibrillation. Quinidine, which blocks I_{to}, is one of the therapeutic candidates for this disorder. In the previous reports, quinidine has been shown to be effective in restoration of loss of the action potential dome, normalization of the ST-segment elevation, and prevention of ventricular tachyarrhythmia [3-5].

Late potentials are frequently detected by signal-averaged ECG in Brugada syndrome and have been reported to correlate to episodes of ventricular tachyarrhythmia [7]. In Brugada syndrome, presence of late potentials is considered to be related to (1) prolonged action potential with second upstroke and/or phase 2 reentry between the epicardial sites with different action potential duration in the right ventricular outflow tract and (2) delayed conduction in some of the area in the right ventricle [3,9-12]. Although precise mechanism was uncertain, the elimination of late potentials by quinidine in our case may be responsible for responsible for restoration of phase 1 action potential notch and reappearance of phase 2 dome by I_{to} blocking property of quinidine.

It has been reported that late potentials become more prominent by flecainide and pilsicainide, sodium current blockers, that can exacerbate ST-segment elevation in Brugada syndrome [13,14]. Although quinidine also has sodium current–blocking properties, the effects on I_{to} blocking may be relatively stronger and can eliminate late potentials in this patient.

Quinidine did not alter the ST-segment elevation or its morphology. Indeed, in the previous study, no correlation has been observed between the presence of late potentials and the magnitude of J-point elevation [7]. Moreover, quinidine has been reported to prevent ventricular tachyarrhythmic events without favorable changes of the ST-segment elevation [4].

Quinidine prolonged the QT interval but not the QRS interval in the present patient. The blood concentration of quinidine was at lower limit of the therapeutic range (2-5 μg/mL), and the effects of quinidine in blocking the sodium current appeared weak or negligible. However, the effects on I_{to} and/or other potassium channels may have been sufficient to prolong the QT interval and eliminate late potentials.

The day-to-day or circadian variation of ST-segment elevation in body-surface ECG is well known in Brugada syndrome [6,15]. However, such spontaneous changes of late potentials have not been well studied. Although the