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EXPERIMENTAL STUDY

Autoimmunity Against the Second Extracellular Loop of Beta$_1$-Adrenergic Receptors Induces Early Afterdepolarization and Decreases in K-Channel Density in Rabbits

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OBJECTIVES We sought to define the electrophysiologic property of the rabbit heart associated with autoimmunity against the second extracellular loop of the beta$_1$-adrenergic receptor.

BACKGROUND Sudden death of patients with cardiomyopathy, probably due to lethal ventricular arrhythmias, can be predicted by the presence of autoantibodies against the second extracellular loop of the beta$_1$-adrenergic receptor.

METHODS Rabbits were immunized by repetitive subcutaneous administration of a synthetic peptide corresponding to the second extracellular loop of beta$_1$-adrenergic receptors (beta group; n = 30) for a mean of 4.2 months. Control rabbits received only vehicle (control group; n = 30).

RESULTS One of the rabbits in the beta group died suddenly during the observation period, but none of the control animals died. The prevalence of sustained ventricular tachycardia was significantly higher in the beta group (beta: 4 of 27 vs. control: 0 of 30), and a standard microelectrode experiment revealed prolongation of the action potential duration (APD) in the right ventricular papillary muscle (beta: 156 ± 5 ms vs. control: 131 ± 4 ms; $p < 0.05$). Early afterdepolarization (EAD) was observed in one rabbit in the beta group (1 of 26), but not in any animals in the control group (0 of 17). A dose of 100 nmol/l of E-4031 induced EAD in the beta group (10 of 10), but not in the control group, except for one rabbit (1 of 10). The whole-cell, patch-clamp experiment on left ventricular M cells showed significant decreases in transient outward current (I_{to1}) (−43%) and slowly activated delayed rectifier current (I_{Ks}) densities (−33%), whereas the inward-rectifying K current (I_{K1}) and rapidly activated delayed rectifier current (I_{Kr}) densities remained unchanged.

CONCLUSIONS Long-term immunization against the second extracellular loop of the beta$_1$-adrenergic receptor caused EAD and APD prolongation and decreased the K-channel density, suggesting that an arrhythmic substrate via autoimmune mechanisms is present in cardiomyopathic patients who have autoantibodies directed against the receptors. (J Am Coll Cardiol 2004;43:1090–100) © 2004 by the American College of Cardiology Foundation

Cardiomyopathy is often complicated by ventricular arrhythmia and sudden cardiac death (1–3), and the electrocardiographic (ECG) changes frequently observed in these patients (i.e., ST-segment and T-wave abnormalities [4,5]) suggest some underlying ion channel disorders that might be the cause of such lethal arrhythmias.

Autoantibodies produced against the beta$_1$-adrenergic receptor can be detected in 30% to 50% of patients with idiopathic cardiomyopathy (6–9), but only a subgroup of autoantibodies directed against the second extracellular loop of the receptors has been found to exert sustained, intrinsic sympathomimetic-like actions on cardiomyocytes in vitro (7). Autoantibody against this domain was found in 38% of the patients with idiopathic dilated cardiomyopathy in our previous study (10), and the incidences of ventricular tachycardia (VT) and sudden death were higher in patients with such autoantibodies than in those without them, despite the absence of any difference in cardiac function or neurohormonal levels. This autoantibody is still the sole hormonal factor that has been clearly shown to be related to sudden death and sustained VT in cardiomyopathic patients. Although the autoantibody has been demonstrated to induce an acute increase in L-type calcium current (I_{CaL}) conductance and action potential duration (APD) prolongation (11), the effect of long-term ion channel stimulation by the autoantibody remains to be elucidated.

Acquired cardiomyopathy is partly associated with autoimmunity, as well as virus infection (12), and in our previous study, autoimmunity against the second extracellular loop of beta$_1$-adrenergic receptors was shown to induce myocardial hypertrophy in the rabbit (13), suggesting that autoantibod-

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Abbreviations and Acronyms

APD = action potential duration
BSA = bovine serum albumin
EAD = early after depolarization
ECG = electrocardiogram/electrocardiograph/electrocardiographic
I_{CaL} = L-type calcium current
I_f = hyperpolarization-dependent current
I_{K1} = inward-rectifying K current
I_{Kr} = rapidly activated delayed rectifier current
I_{Ks} = slowly activated delayed rectifier current
I_{to} = transient outward current
PCL = pacing cycle length
SVT = sustained ventricular tachycardia
VT = ventricular tachycardia

ies to this domain were not only the factor that precipitated sudden cardiac death but also a primary cause of cardiomyopathy. Our autoimmune rabbit model therefore provides an opportunity to characterize the pathophysiology of cardiomyopathy, especially the pathophysiology related to lethal arrhythmias, which are the predominant cause of sudden cardiac death in cardiomyopathic patients.

METHODS

Immunization. Experiments were performed on 60 male Japanese white rabbits at 10 weeks of age (weight 1.8 to 2.2 kg). Immunization was carried out according to the procedure described previously (13). Briefly, a synthetic peptide corresponding to the second extracellular loop of the rabbit's beta$_1$-adrenergic receptor (residues 197 to 222; HWWRAESDEARRCYNDPKCCDFVTNR) was produced by the Peptide Institute Inc. (Osaka, Japan). A group of rabbits was immunized by monthly subcutaneous injection of the peptide (1 mg) dissolved in 1 ml saline (pH adjusted to 4 with HCl), conjugated with 0.5 ml of complete and incomplete Freund's adjuvant (beta group), and 30 control rabbits received saline containing adjuvant in the same manner (control group). All experiments were approved by the ethics committee of Keio University School of Medicine. The mean observation period was 4.2 ± 0.3 months. The observation periods in our pilot study were three and six months, but because there were no differences in electrophysiologic properties between the two groups, we averaged the data in the present study.

Tissue and cell preparations. At the end of the observation period, an ECG was recorded from the limb lead under closed-chest conditions during anesthesia with chloral hydrate, as described previously (13). The ECG could not be recorded in two rabbits in the beta group because of technical problems. After intravenous injection of heparin (2,000 U) and pentobarbital sodium (30 mg/kg), the heart was excised and retrogradely perfused with normal $NaHCO_3$-buffered Tyrode's solution for 5 min to wash out the blood cells. The excised right ventricular papillary muscle was placed in a bath superfused with the $NaHCO_3$-buffered Tyrode's solution at 37°C and paced for few hours before the experiment to equilibrate the recording. Field stimulation was achieved with 2-ms-wide biphasic pulses (2× threshold intensity; SS-102J and EE-602J, Nihon Kohden, Tokyo, Japan) between the Ag-AgCl electrodes on the bath. For technical reasons, we did not record the action potentials of 13 rabbits in the control group and 3 rabbits in the beta group.

Single ventricular myocytes were obtained by enzymatic dissociation from the M-cell region of the left ventricular free wall of the retrogradely perfused heart. Nominally Ca^{2+}-free Tyrode's solution with 0.1% bovine serum albumin (BSA) (fraction V, Gibco, California) was perfused for 3 min and then switched to an enzyme solution containing 0.5 g/l of type II collagenase (Worthington Biochemical, New Jersey) and 0.2% BSA for 20 to 22 min. Next, the left ventricular free wall was isolated, and thin slices of tissue were cut along the surface of the ventricle in the M-cell region (middle one-third of wall) with a dermatome (Davol Simon Dermatome, Rhode Island, power handle no. 3293 with cutting head no. 3295). The tissue was minced, incubated, and gently agitated in enzyme solution containing 0.5 g/l of the collagenase, 3% BSA, and 0.3 mmol/l $CaCl_2$. Incubation with fresh enzyme solution was repeated 5 to 7 times at 10-min intervals. The supernatant from each digestion was filtered (120-μm mesh) and centrifuged (400 rpm for 2 min). The cells were then stored in Tyrode's solution supplemented with 0.5 mmol/l $CaCl_2$ at room temperature for 2 to 7 h before the patch-clamp experiment. Ventricular myocytes were plated on 14-mm-round glass coverslips (Matsunami, Tokyo, Japan) precoated with 150 μl of laminin (20 ml/l; Boehringer, Mannheim, Germany).

Solutions and drugs. The $NaHCO_3$-buffered Tyrode's solution contained (mmol/l): NaCl 129.5, KCl 5, NaH_2PO_4 0.9, $NaHCO_3$ 20, $MgSO_4$ 1.2, $CaCl_2$ 1.8, and glucose 5.5 (pH adjusted to 7.4 at 37°C) with oxygenation (95% oxygen/5% carbon dioxide). Tyrode's solution contained (mmol/l): NaCl 140, KCl 4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, HEPES 5, and glucose 5.5 (pH adjusted to 7.4 with NaOH). Nominally Ca^{2+}-free Tyrode's solution was prepared by simply omitting $CaCl_2$.

The pipette solution for the transient outward current (I_{to}) contained (mmol/l): NaCl 10, KCl 130, EGTA 15, $MgCl_2$ 1, HEPES 5, ATP-Na 5, GTP-Na 0.3, and $CaCl_2$ 7 (pCa calculated as 7; pH adjusted to 7.2 with KOH). For other K channels it contained: KCl 140, HEPES 5, EGTA 10, $MgCl_2$ 1, ATP-Mg 5, and GTP-tris 0.3 (pH adjusted to 7.2 with KOH). To prevent contamination by inward currents, the extracellular Na^+ ion was replaced by equimolar choline, and the Ca^{2+} ion was replaced by Mn^{2+}. Two μmol/l of nisoldipine was used to block the residual Mn^{2+} current via I_{CaL}. The details of the extracellular solution used in the experiments are shown in Table 1. The pipette solution for I_{CaL} contained (mmol/l): CsCl 115, TEACl 20, BAPTA 10, HEPES 5, ATP-Mg 3, and GTP-tris 0.4 (pH was adjusted to 7.2 with CsOH).

Table 1. The Extracellular Solutions Used in the Experiments

Solution	I_{to}	I_{Kr}	I_{Ks}	I_{K1} and I_f
KCl	4	6	0	4
NaCl				135
CholineCl	135	135	135	
$MnCl_2$	1.8	1.3	1.8	1.8
$BaCl_2$		0.5		
$MgCl_2$	0.5	0.5	0.5	0.5
Glucose	5.5	5.5	5.5	5.5
HEPES	5	5	5	5
Chromanol-293b		0.001		
E-4031			0.001	
Ouabain				0.003
TTX				0.001

Data are expressed as mmol/l.

I_f = hyperpolarization-dependent current; I_{K1} = inward-rectifying current; I_{Kr} = rapidly activated delayed rectifier current; I_{Ks} = slowly activated delayed rectifier current; I_{to} = transient outward current; TTX = tetradotoxin.

Most chemicals were obtained from Sigma-Aldrich (St. Louis, Missouri) and Wako Chemicals (Tokyo, Japan). Ouabain (Sigma-Aldrich), nisoldipine (Bayer, Leverkusen, Germany), 4-aminopyridine (Sigma-Aldrich), E-4031 (Eisai, Tokyo, Japan), tetrodotoxin (TTX, Sankyo, Tokyo, Japan), bisoprolol (Tanabe, Osaka, Japan), and chromanol-293b (Aventis, Frankfurt, Germany) were freshly prepared before each use. Nisoldipine and chromanol-293b were dissolved in dimethyl sulfoxide and then diluted to the extracellular solution. The final concentration of dimethyl sulfoxide was <0.1%.

Recording techniques. The transmembrane action potential was recorded with glass microelectrodes filled with 2.7 mol/l KCl (10 to 20 MΩ of pipette resistance) connected to a high-input impedance amplifier (MEZ-8300, Nihon Kohden). The amplified signal was filtered with a four-pole Bessel filter (NF-3625, NF electronic instrument, Tokyo, Japan) set at 2 kHz, then digitized with an A/D converter (PCI-MIO-16E4, National Instruments Japan, Tokyo, Japan) at a sampling frequency of 10 kHz and stored in a personal computer (Macintosh G3, Apple Computer, Tokyo, Japan) with Igor Pro 4 and NIDAQ tool commercial software (Wavemetrics Inc., Lake Oswego, Oregon). The maximum diastolic potential, maximum positive deflection of the phase 0 upstroke, amplitude of the action potential, and APD at 50% and 90% repolarization (APD_{50} and APD_{90}) were measured. Chemical exposure was via the superfusate, and measurements were made after waiting at least 30 min for stabilization.

Whole-cell K currents and I_{CaL} were measured by standard whole-cell, patch-clamp techniques. Cardiomyocytes were transferred into a thermo-controlled perfusion chamber (37°C to 37.5°C) mounted on the stage of an inverted microscope (IX-70, Olympus, Tokyo, Japan) and superfused with Tyrode's solution. Superfusates were applied via a gravity-fed thermo-controlled Y-tube with a diameter of approximately 120 μm. Its opening was positioned approximately 150 μm from the cell. The bath temperature and heater temperature of the Y-tube were monitored with a digital thermistor (model 2455, Iuchi, Osaka, Japan). The resistance of pipettes filled with internal solution was 1.2 to 1.8 MΩ, and the pipettes were coupled to an amplifier via an Ag-AgCl wire. The liquid junction potentials at the recording pipette and the ground electrode were −1 to −2 mV. Seal resistances <4 GΩ, and series resistances >2 MΩ were discarded from the analysis. Membrane voltages were computer-controlled (pClamp-8 software, Axon Instruments Inc., Union City, California). The currents were amplified and then filtered with a built-in four-pole Bessel filter set at 10 kHz (Axopatch-200B, Axon Instruments Inc.). Data were sampled with an A/D converter (DigiData-1321A, Axon Instruments Inc.) at a frequency of 10 kHz and stored in an AT/T computer. Recording was started 3 min after rupturing the patch membrane in order to allow the contents of the pipette and the cytoplasm to equilibrate.

To measure I_{to}, we held cells at a potential of −80 mV before evoking a 10-ms conditioning pulse to −50 mV to inactivate the fast sodium current and T-type calcium current and then applied a 300-ms test pulse from −20 to +70 mV in 10-mV increments. We set the sweep-to-sweep interval at 30 s to ensure complete recovery of I_{to}. The capacitance current and other background currents were elicited by the same step-test-pulse and conditioning-pulse protocol, but from the holding potential of 0 mV to inactivate I_{to}, and then subtracted from the previous data. The remaining data were defined as the I_{to}. In some experiments, the pulse-to-pulse interval was changed from

Table 2. Animal Characteristics and Electrophysiologic Data of Rabbits in the Control and Beta Groups

	Control Group (n = 30)	Beta Group* (n = 29)
Body weight (kg)	3.2 ± 0.1	3.3 ± 0.1
Sudden death	0/30	1/30
ECG data		
PQ interval (ms)	59.7 ± 1.7	58.0 ± 2.0
QRS duration (ms)	43.5 ± 2.1	45.4 ± 2.3
RR interval (ms)	195.4 ± 4.1	177.3 ± 3.7†
QRS axis (degree)	19.5 ± 20.4	44.4 ± 20.2
QTc ($ms^{1/2}$)	305.0 ± 6.7	314.6 ± 5.4
QT/RR interval ratio	0.691 ± 0.016	0.751 ± 0.017†
	n = 30	n = 27
Action potential (PCL = 2 s)		
APD_{90} (ms)	131 ± 4	156 ± 5†
APD_{50} (ms)	82 ± 4	106 ± 6†
Amp (mV)	112 ± 2	112 ± 2
RMP (mV)	−88.2 ± 0.5	−89.3 ± 0.4
V_{max} (V/s)	116 ± 8	117 ± 8
Early afterdepolarization	0/17	1/26
	n = 17	n = 26

*Beta denotes rabbits immunized by repetitive subcutaneous administration of a synthetic peptide corresponding to the second extracellular loop of beta$_1$- adrenergic receptors. Control rabbits received vehicle only. †$p < 0.05$ versus control group. Data are presented as the mean value ± SE.

Amp = amplitude of action potential; APD_{50} and APD_{90} = action potential duration at 50% and 90% repolarization, respectively; ECG = electrocardiographic; PCL = pacing cycle length; QTc = corrected QT interval; RMP = resting membrane potential; V_{max} = maximum positive deflection of phase 0 upstroke.

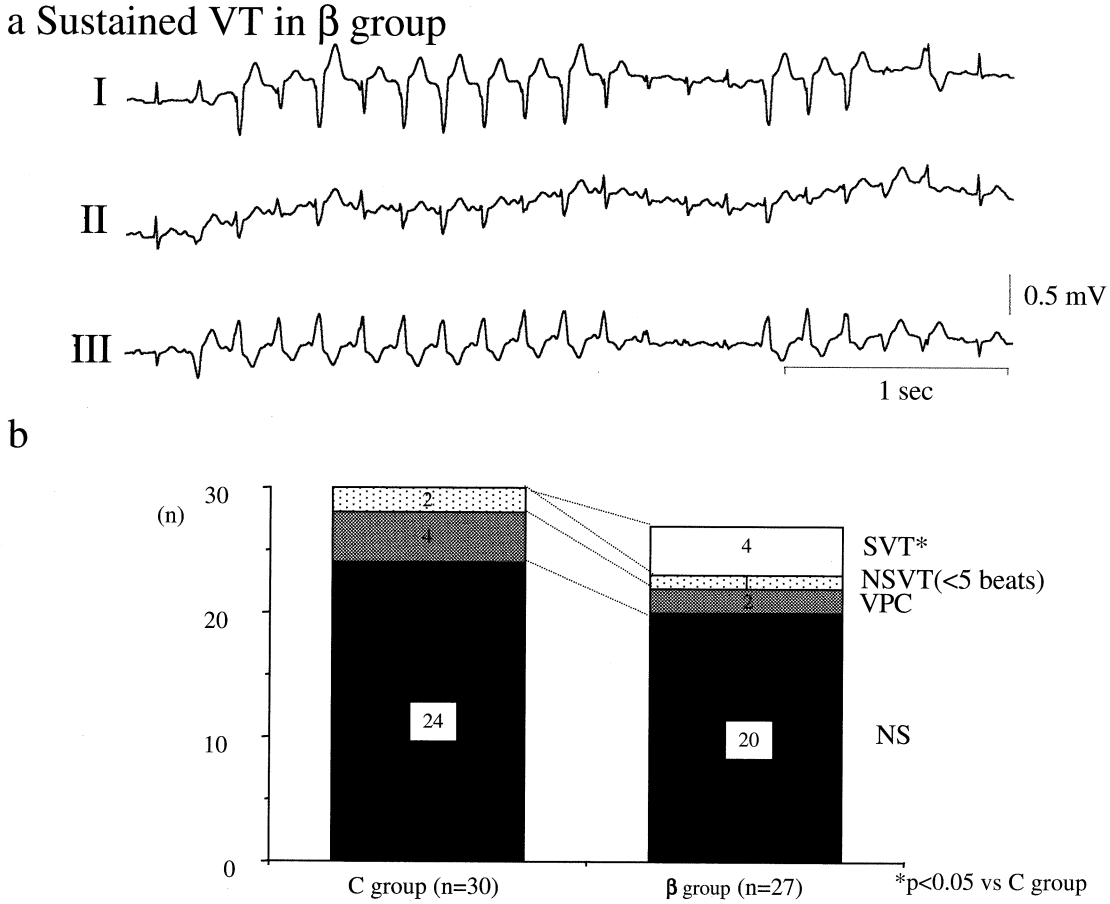

Figure 1. **(a)** Representative trace of sustained ventricular tachycardia (SVT) on electrocardiograms from the beta group of rabbits. Wide QRS tachycardia (maximum 13 beats) was seen. **(b)** Summary of the ventricular arrhythmias observed on the electrocardiogram. The prevalence of SVT was significantly higher in the beta group than in the control group. C = control; NS = normal sinus rhythm; NSVT = non-sustained ventricular tachycardia; VPC = ventricular premature contractions.

0.5 to 30 s, and the I_{to} density evoked by the 300 ms to +70 mV test pulse from the holding potential of −80 mV with the 10-ms conditioning pulse to −50 mV was measured. The I_{to} density at the 50th test pulse was measured to observe the rate adaptation. Slowly activated delayed rectifier (I_{Ks}) and rapidly activated delayed rectifier (I_{Kr}) were defined as 1 μmol/l of E-4031-insensitive current and 1 μmol/l of chromanol-293b-insensitive current, respectively (14,15). These doses of compound caused specific and complete blockade of the current (14,15). The currents were measured as the amplitude of the tail current after the depolarization test pulse. The cells were held at a potential of −40 mV before evoking a 5-s (I_{Ks}) or 500-ms (I_{Kr}) test pulse from −40 to +60 or +70 mV in 10-mV increments, and then returned to −40 mV again. The inward-rectifying K current (I_{K1}) and hyperpolarization-dependent current (I_f) were measured by the hyperpolarizing step pulse. We held cells at −60 mV before evoking a 1-s test pulse from −40 to −150 mV in 10-mV increments. To measure I_{CaL}, we held cells at a potential of −80 mV before evoking a 300-ms conditioning pulse to −50 mV to inactivate the T-type calcium current and then applied a 300-ms test pulse from −40 to +60 mV in 10-mV increments. We set the sweep-to-sweep interval at 30 s to ensure complete recovery of I_{CaL}.

Cell capacitance was calculated by the 5 mV of ±1 V/s ramp pulse immediately before each voltage-clamp protocol. Currents obtained in the present study were normalized to each cell capacitance.

Measurements and statistical analysis. All data are shown as the mean value ± SE. The statistical significance of the difference between the mean values was estimated by the Student *t* test. Differences were considered significant at $p < 0.05$. The Fisher exact probability test was used to test the significance of differences in the incidence of arrhythmia between groups. Ventricular rhythm was classified as follows: normal sinus rhythm; ventricular premature contraction; non-sustained VT (<5 beats); and SVT = sustained ventricular tachycardia (≥5 beats). The tangent of the T wave was extended to the baseline to define the end of the T-wave. The corrected QT interval was calculated by Bazett's formula.

RESULTS

One rabbit in the beta group died suddenly during observation period (at two months). None of the rabbits in the

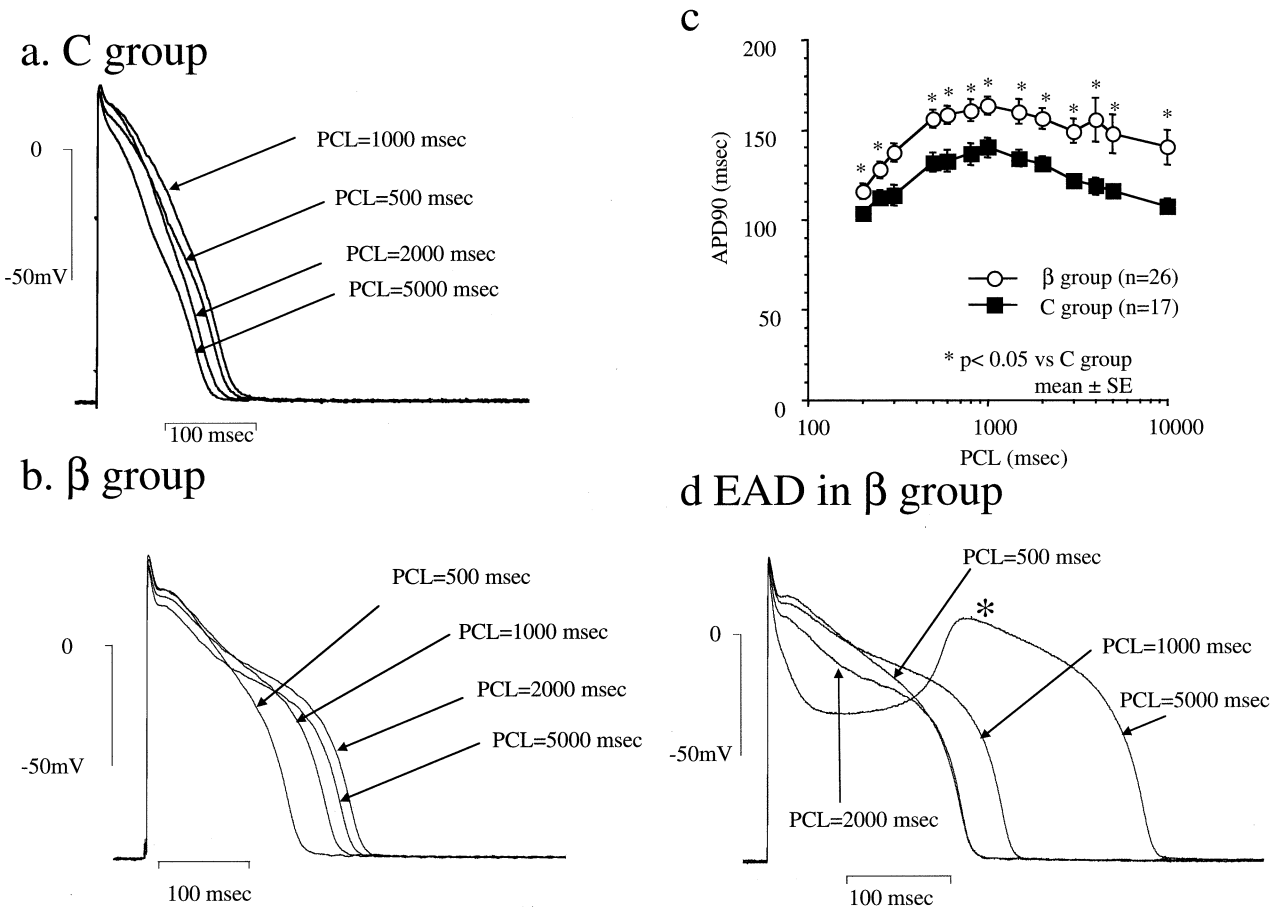

Figure 2. Superimposed representative action potentials recorded from the right ventricular papillary muscle of rabbits in the control group **(a)** and beta group **(b)** at various pacing cycle lengths (PCLs). Retardation of repolarization around membrane potentials of −10 to −20 mV and prolongation of action potential duration (APD), especially at the lower PCL, were observed in 46% of the beta group. **(c)** Action potential durations measured at 90% repolarization (APD_{90}) were averaged and plotted against the PCL on a semi-log scale. In the control group, APD_{90} shortened as a function of a shorter PCL (<1,000 ms), whereas at >2,000 ms, it shortened as a function of a longer PCL. The APD_{90} of the beta group of rabbits was significantly longer at all PCLs, compared to the control group rabbits, and the degree of the prolongation was marked at longer PCLs. **(d)** The action potential of one of the beta group rabbits showed early afterdepolarization **(*)**, which was more prominent at slower PCLs. C = control.

control group died during the observation period. Autopsy did not reveal any cause of death, suggesting that sudden cardiac death due to some lethal arrhythmia may have been the cause. The body weights of the animals at the end of the observation period were similar (Table 2). The heart rate was significantly higher in the beta group than in the control group. The corrected QT interval increased slightly in the beta group, but the difference from baseline was not statistically significant in both groups. The QT/RR interval ratio was significantly higher in the beta group than in the control group (Table 2). Sustained VT was more frequently noted in the beta group than in the control group (Fig. 1).

Action potential recording from right ventricular papillary muscle. Standard microelectrode experiments showed significant prolongation of APD_{90} and APD_{50} in the beta group at the 2,000-ms pacing cycle length (PCL), compared with the control group, but there were no differences in any other parameters measured (Table 2). The APD prolongation in the beta group was observed at PCLs between 200 and 10,000 ms, compared with the control group, and it was more prominent at slower PCLs than at faster PCLs (Fig. 2c). The action potential showed prolongation and a hump at phase 3 around membrane potentials from less than −10 to −20 mV (Fig. 2b), especially at the slower PCLs in the beta group, but not in the control group (Fig. 2a). Early afterdepolarization (EAD) was observed in one rabbit in the beta group (Fig. 2d [see asterisk]) at the slower PCLs (>2,000 ms). The residual serum autoantibodies in the tissue sample may have stimulated the beta$_1$-adrenoceptor and caused APD prolongation via increasing conductance of I_{CaL}. To rule out this possibility, we administered 1 μmol/l bisoprolol as a selective beta$_1$-adrenoceptor blocker, but it failed to reverse the APD prolongation in the beta group (Fig. 3) (we tried 10 μmol/l in some experiments, but obtained the same results [data not shown]). These findings suggest that APD prolongation may reflect underlying electrical remodeling in ion channels instead of a direct agonistic action of the autoantibodies. A dose of 100 nmol/l of E-4031 was applied in some experiments, and EAD and APD prolongation were observed. A significantly higher

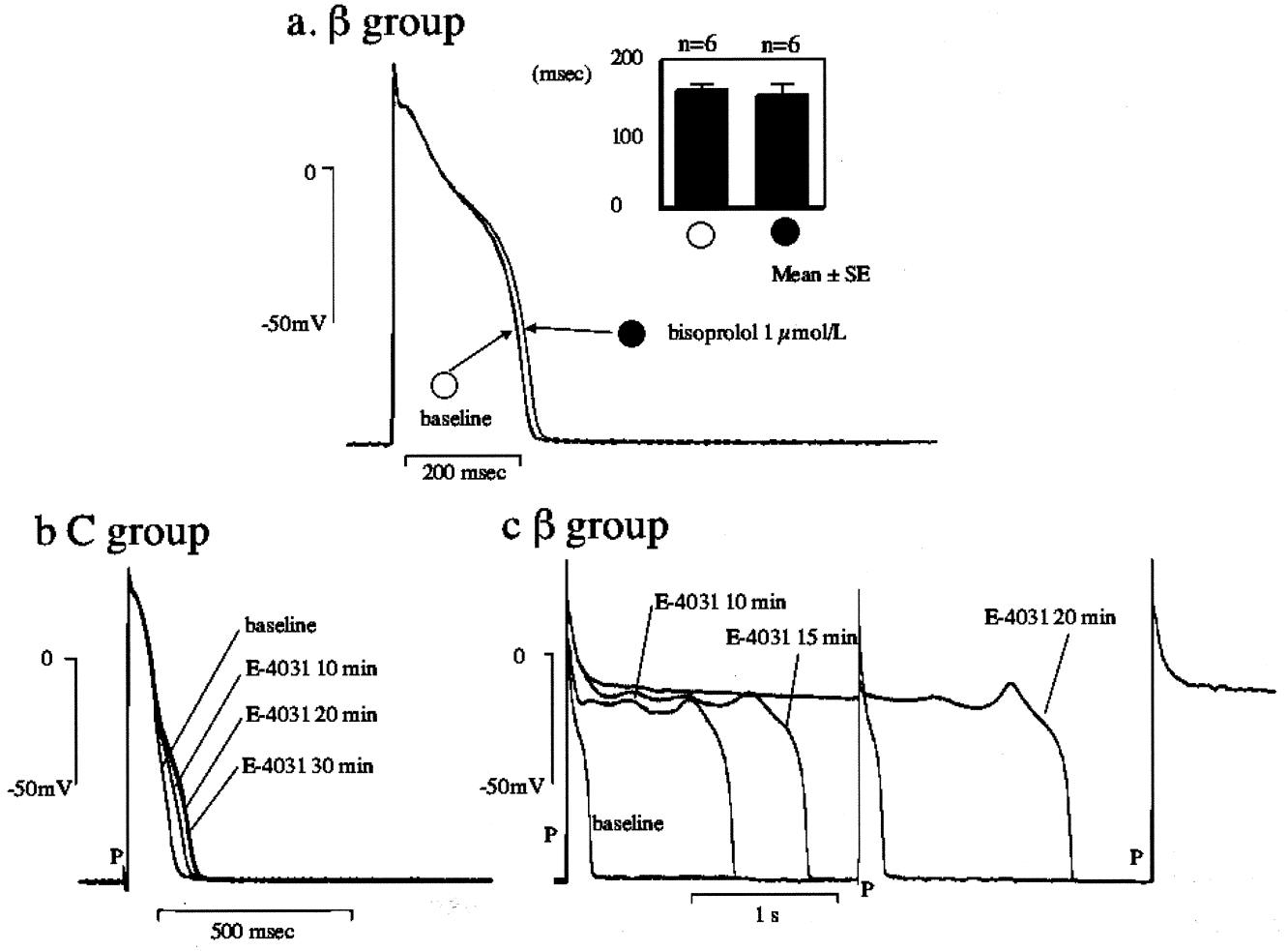

Figure 3. **(a)** Effect of bisoprolol on action potential duration (APD) in the right ventricular papillary muscle of rabbits in the beta group. Representative action potentials at a pacing cycle length of 2,000 ms, obtained from beta group rabbits before and after administration of 1 μmol/l of bisoprolol, have been superimposed. In the inset, values of APD at 90% repolarization (APD_{90}), measured before and after administration of bisoprolol, were averaged. Bisoprolol did not affect the prolonged APD in the beta group. Representative E-4031 (100 nmol/l) effects on the action potentials in the control **(b)** and beta groups **(c)** are shown. Slight prolongation in APD was observed in the control group, but it was not early after depolarization. On the other hand, E-4031 induced significant APD prolongation in the beta group and finally caused pacing failure at 20 min after administration. C = control; P = pacing artifact.

rate of E-4031-induced EAD was observed in the beta group (10 of 10) than in the control group (1 of 10) (Fig. 3b). Pacing at 2,000-ms PCL was impossible in two rabbits in the beta group because of marked prolongation of the action potential (Fig. 3c).

Whole-cell, patch-clamp experiment from isolated ventricular myocytes. The myocytes isolated from both groups appeared similar. There was no difference in cell capacitance between the control group (178.0 ± 5.7 pF) and the beta group (188.9 ± 5.6 pF). The I_{to} was completely blocked by 1 mmol/l 4-aminopyridine, suggesting that the I_{to} recorded in the present study mainly consisted of I_{to1} (16). The I_{to} density was significantly smaller in the beta group than in the control group (Fig. 4), but the current-voltage relationship was similar. The rate-dependent change in I_{to} density was also similar (Fig. 4d). The tail of I_{Ks} density was significantly smaller in the beta group than in the control group (Fig. 5), without changing the current-voltage relationship. There was no difference in the I_{Kr} density or current-voltage relationship (Figs. 6a to 6c). The I_{K1} density tended to be greater at the lower membrane potential in the beta group, but the difference was not statistically significant. The current-voltage relationship and outward component of I_{K1} were similar (Fig. 6d). No I_f was present in either group. There was no difference in the peak I_{CaL} density (control group: −12.0 ± 0.3 pA/pF [n = 7]; beta group: −12.1 ± 1.0 [n = 11]; p = NS).

DISCUSSION

Our major novel findings were: 1) electrical remodeling caused by autoimmunity against the second extracellular loop of beta$_1$-adrenergic receptor in ventricular myocytes (i.e., APD prolongation and EAD) and a decrease in I_{Ks} and I_{to1} density in the beta group; and 2) an increase in the incidence of SVT in the beta group. One rabbit in the beta group died suddenly during the observation period, and there was no clear cause of death.

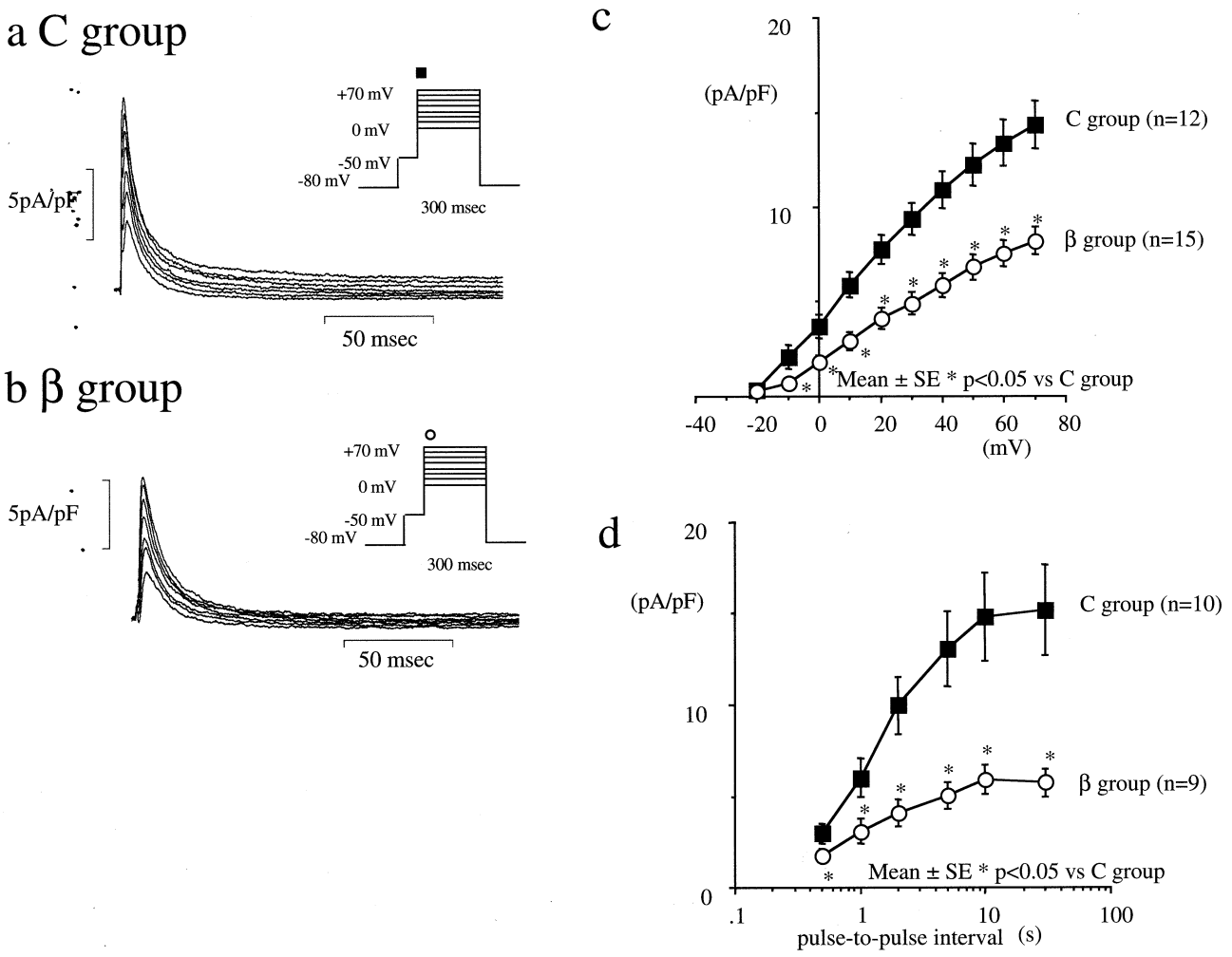

Figure 4. Representative current traces of I_{to} in rabbits of the control **(a)** and beta groups **(b)**. The I_{to} elicited by the voltage-clamp protocol in the inset was normalized to the cell capacitance. The measured maximum outward current was averaged and plotted against the test potential **(c)**. The I_{to} density obtained from the beta group rabbits was significantly smaller than that from control group rabbits. The I_{to} density, elicited by the test pulse to +70 mV at the various pulse-to-pulse interval, is shown in **(d)**. See text for details. (Data were obtained from isolated left ventricular myocytes from the M-cell region). C = control.

Autoantibody characterization and rabbit model. Autoantibody against the second extracellular loop of the beta$_1$-adrenergic receptor is one of the putative antibodies that may cause arrhythmogenesis and sudden cardiac death in patients with idiopathic cardiomyopathy (10). Autoantibody to that domain blocks the intrinsic sympathomimetic action of catecholamines, while exerting a partial agonist action on the beta$_1$-adrenergic receptor (7,13). The Western blot analysis in our previous study showed the absence of any cross-reaction between the autoantibody and other cardiac proteins (13). In contrast to typical adrenergic agonists, such as isoproterenol, the autoantibody does not induce desensitization of the receptor, and this may maintain long-term sympathomimetic stimulation (7).

The autoantibodies induced cardiac hypertrophy in rabbits with predominantly diastolic dysfunction over time, which was prevented by a selective beta$_1$-adrenergic receptor blocker (13), suggesting that the changes were not attributable to the nonspecific inflammatory action of the adjuvant but to a specific beta$_1$-adrenergic receptor-stimulating action of the autoantibody. Our autoimmune rabbit model did not exhibit left ventricular dilation; however, we speculate that a longer period of immunization may have caused ventricular dilation, because 10% to 15% of hypertrophic cardiomyopathic patients exhibit left ventricular dilation and dysfunction (17) in the end stage of the natural history of their disease. No dilation of the left ventricle was observed at the end of the 12-month observation period in our previous study; however, regression in hypertrophy was present (13).

In the present model, myocardial hypertrophy and interstitial fibrosis were observed at six months after immunization, but never by three months (13). This finding is interesting because the electrophysiologic change preceded the histologic change. The cell capacitance did not change between the two groups, even in the subgroup of rabbits that

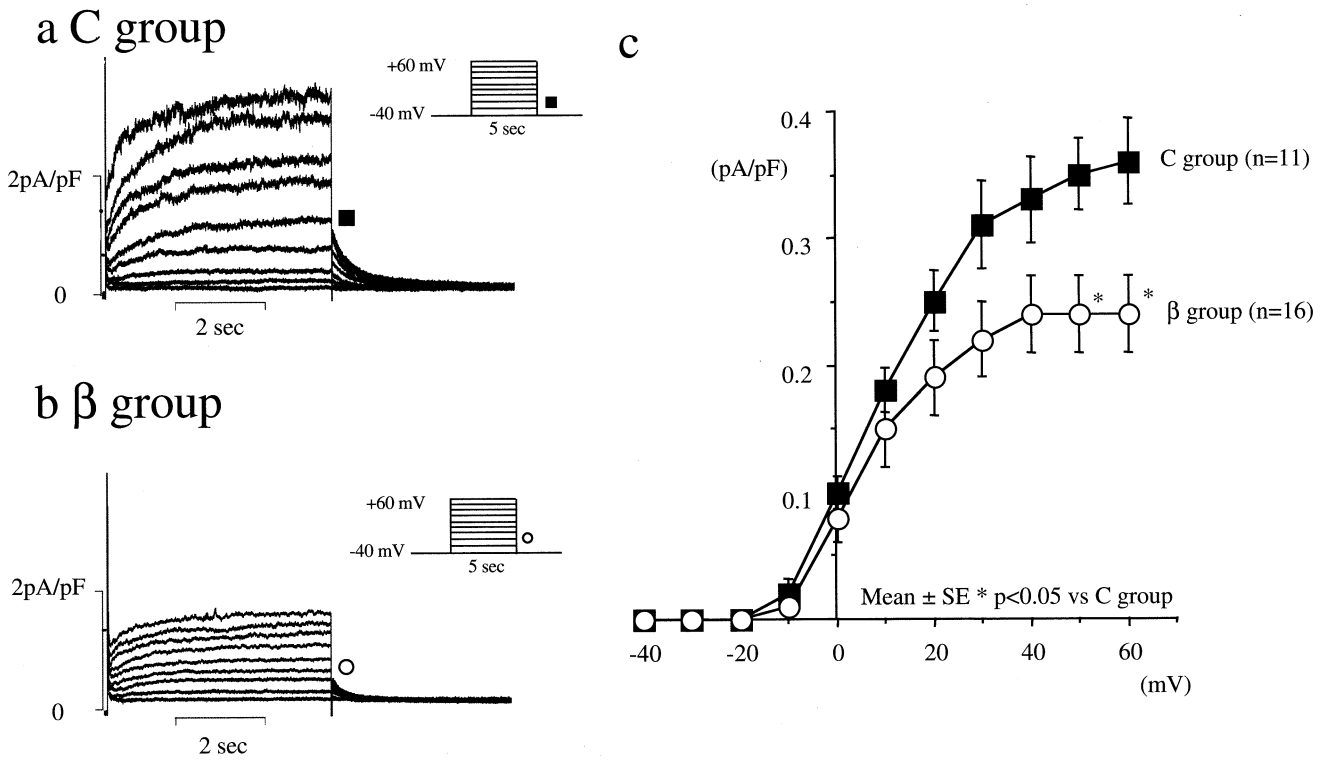

Figure 5. Representative current traces of the E-4031-insensitive current, as I_{Ks}, in rabbits of the control **(a)** and beta groups **(b)**. The I_{Ks} elicited by the voltage-clamp protocol in the inset was normalized to the cell capacitance. Measured maximum outward tail currents were averaged and plotted against the test potential **(c)**. The I_{Ks} density obtained in the beta group rabbits was significantly smaller than that in the control group rabbits. (Data were obtained from isolated left ventricular myocytes from the M-cell region.) C = control.

was immunized of six months. Because cell capacitance does not necessarily reflect cell volume or hypertrophy itself, only the membrane surface area, we speculate that our model may not undergo any increase in membrane surface area until at least six-month immunization.

Electrophysiologic change. Because Bazett's formula might not be valid for correction of QT intervals when the RR interval is extremely short, we used the QT/RR ratio as an indicator of the QT interval. The APD prolongation, EAD, and coupled decrease in K-channel density in vitro may be responsible for the QT prolongation, sudden death, and SVT in vivo, and that would be consistent with our clinical observations in patients with dilated cardiomyopathy who have autoantibodies against the domain (10). The direct sympathomimetic-like actions of the autoantibodies (11) may not play a major role in the electrophysiologic properties in vivo.

Because chemical mediators and/or autoantibodies in blood vessels may affect the electrophysiologic properties of the papillary muscle, blood was completely flushed out in the present study (18). There was no difference between the plasma and left ventricular norepinephrine concentrations in the two groups in the present model (13). Furthermore, 1 μmol/l bisoprolol, which is known to inhibit the sympathomimetic-like action of autoantibody against this domain (7), was incapable of reversing the APD prolongation. This suggests that APD prolongation was not caused by the direct sympathomimetic-like action of the residual antibody in the tissue sample, but by electrical remodeling of the channel.

Functional downregulation of K currents has been observed in a variety of models of hypertrophied and failing ventricular myocardium. A reduction in I_{to} density is the most consistent ionic current change in the model of cardiac hypertrophy and failure, including the pacing-induced heart failure rabbit model (19) and failing human heart (20), and our findings in this study are consistent with these previous findings in this regard. The influence of the decreased I_{to} on APD, however, is still unclear (20,21). Furthermore, studies of the I_K in the failing heart have been limited and confusing. Studies of cells isolated from the ventricles of pressure-overloaded guinea pigs (22) and spontaneously hypertensive rats (23) have demonstrated no change in I_K density. On the other hand, I_{Ks} and I_{Kr} densities were found to be decreased in the pacing-induced heart failure model (19), as well as in the complete atrioventricular block model (24,25). However, in the present study, the I_{Ks} density was decreased, whereas the I_{Kr} density was unaltered. The inconsistency in the results may be explained by different etiologies and the pathophysiology of heart failure.

In the present study, there was no difference in I_{CaL} density between the two groups. We have also observed desensitization of beta$_1$-adrenergic receptor in the beta group, and sera from the beta group did not increase I_{CaL} in

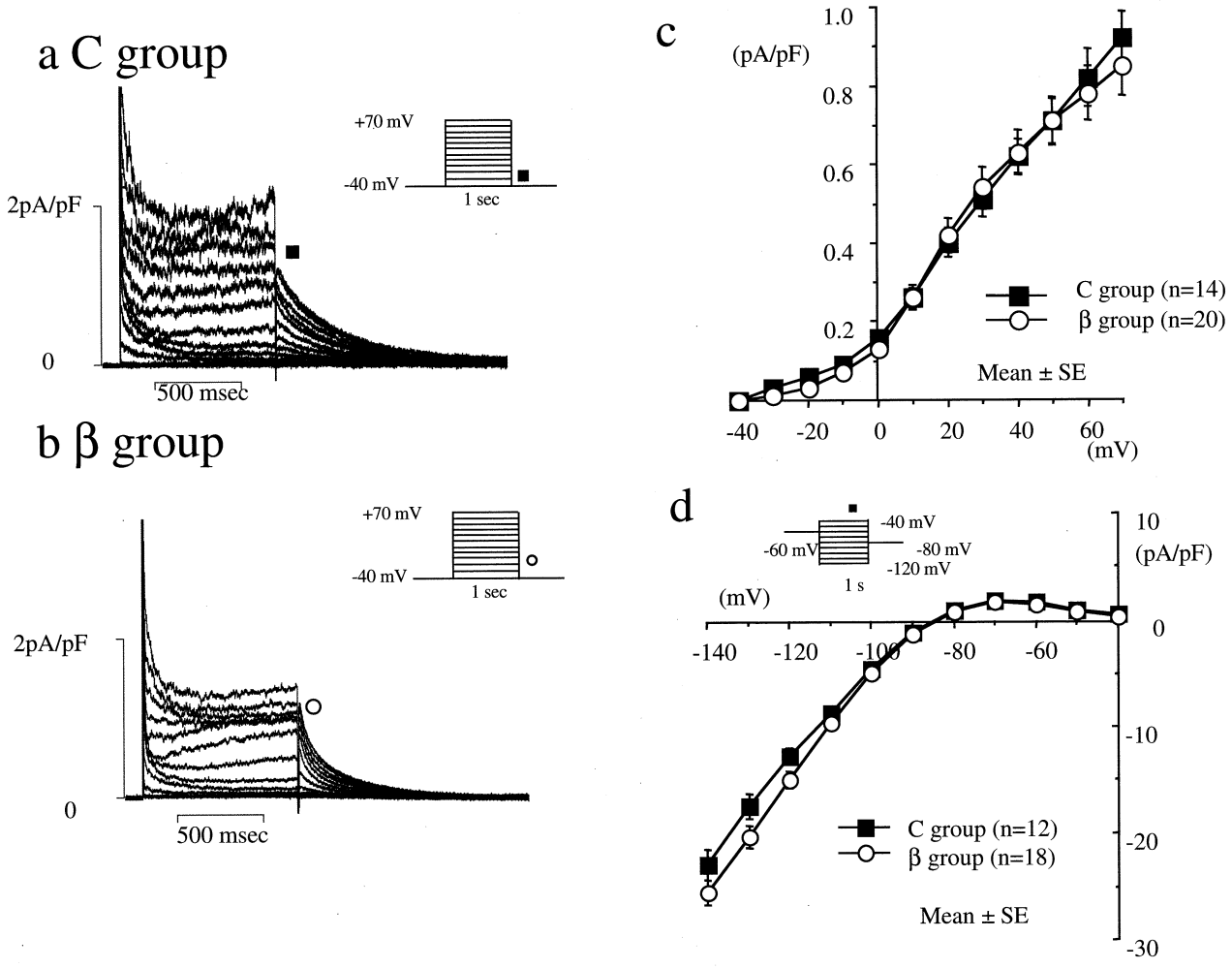

Figure 6. Representative current traces of chromanol-293b-insensitive current, as I_{Kr}, in rabbits of the control **(a)** and beta groups **(b)**. The I_{Kr} elicited by the voltage-clamp protocol in the inset was normalized to the cell capacitance. **(c)** Measured maximum outward tail currents were averaged and plotted against the test potential. There was no difference between the beta group and control groups. **(d)** Measured amplitudes of I_{K1} elicited by the voltage-clamp protocol in the inset were averaged and plotted against the test potential. The currents were normalized to the cell capacitance. There was no statistically significant difference between the two groups in the current–voltage relationship. (Data were obtained from isolated left ventricular myocytes from the M-cell region.) C = control.

the myocytes from the beta group (preliminary observation). Therefore, I_{CaL} density does not play an important role in APD prolongation and EAD. Moreover, APD prolongation was observed at the endocardial surface of the right ventricular papillary muscle, where there is no I_{to} (26), and thus decreased I_{to} density might not be an essential change in this APD prolongation and EAD activity. Decreased I_{Ks} density, which is known to be a major outward current in the repolarization phase, may contribute to APD prolongation and the genesis of EAD, resulting in lethal ventricular arrhythmias in the beta group. As in patients with long QT syndrome type 1, who have I_{Ks} and decreased conductance (27), beta$_1$-adrenoreceptor stimulation may precipitate ventricular fibrillation, as it stimulated both I_{Ks} (28) and I_{CaL}. The inward shift of the net current upon repolarization, especially under conditions of sympathetic stimulation, may precipitate triggered activity and subsequent lethal ventricular arrhythmias in cardiomyopathic patients.

On the other hand, these results suggest the importance of I_{Kr} in the repolarization of the action potential in cardiomyopathic patients. Hypokalemia, which is often observed in patients with heart failure during treatment with loop diuretics, decreases the I_{Kr} conductance and may trigger EAD (29,30). In the present study, a small amount of E-4031 significantly increased the occurrence of EAD in the beta group. In view of this, spironolactone may be a suitable diuretic agent for the prevention of ventricular arrhythmias in cardiomyopathic patients, as suggested by the results of a clinical trial (31). Furthermore, care is required with respect to drugs that block I_{Kr} (i.e., histamine blockers [32,33], erythromycin [34], tricyclic antidepressants [35]) in cardiomyopathic patients who have autoantibodies against the second extracellular loop of beta$_1$ adrenergic receptors.

In the present study, I_{CaL} density did not change between the two groups; however, in our preliminary observation, the

autoantibody increased I_{CaL} (11). Therefore, we speculate that at the beginning of the immunization, an increase in intracellular Ca^{2+} concentration via the increase in I_{CaL} must occur, which may stimulate intracellular Ca^{2+}-dependent signal transduction. Subsequently, ionic channel remodeling might be observed.

Study limitations. The APD prolongation and incidence of ventricular arrhythmia in the present study were modest. The APD prolongation and EAD are protected by activation of I_{Kr} and I_{Ks}, and thus a decrease in I_{Ks} density alone might not cause a marked abnormality in repolarization, as the I_{Kr} density remained unchanged. For example, even if the I_{Ks} density were decreased, the decreased I_{Ks} density would depolarize the membrane potential at the plateau, thereby activating I_{Kr} more, and as a result, the total outward current generated by the delayed rectifiers might be unchanged. However, under the special condition previously mentioned (i.e., hypokalemia) or in the presence of sympathetic stimulation, the I_{Ks} abnormality might trigger EAD and a serious ventricular arrhythmia.

The rapid heart rate of the rabbit may mask the prolongation of the QT interval and incidence of the ventricular arrhythmia in vivo, because APD prolongation was more marked at the slower heart rate in vitro. The mechanism of APD shortening might be the slow kinetics of recovery from inactivation of I_{CaL} and accumulation of I_{Kr} at the rapid heart rate. Furthermore, the low incidence of arrhythmia in the present study might be explained by a loss of transmural dispersion of repolarization via decreasing I_{Ks} density, which might have an anti-arrhythmic effect to some extent (14,27).

Although our observation period was relatively short compared with the natural history of clinical cardiomyopathy, because no relationship between the degree of change in the electrophysiologic property and observation period was found in the present study, we concluded that an essential electrophysiologic change precedes the histologic change. Furthermore, because we did not administer monoclonal autoantibody, the epitope of the autoantibody toward the beta$_1$-adrenergic receptor in the beta group may have been different.

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