Long-Term Endothelin A Receptor Blockade Inhibits Electrical Remodeling in Cardiomyopathic Hamsters

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**Background**—The endothelin (ET) system is activated in failing hearts. Congestive heart failure frequently is associated with ventricular arrhythmias, which may result from electrical remodeling such as changes of ionic current density and heterogeneous action potential prolongation. We examined the effects of long-term ET_A receptor blockade on the electrophysiological properties of ventricular cells, the surface ECG, and the survival in BIO 14.6 cardiomyopathic hamsters.

**Methods and Results**—Membrane currents and action potentials were recorded from left ventricular cells isolated from normal F1β hamsters and cardiomyopathic BIO 14.6 hamsters untreated and chronically treated with TA-0201, an ET_A receptor antagonist. In ventricular cells of untreated BIO 14.6 hamsters, the action potential duration was prolonged and the densities of the L-type Ca2⁺ current (I_{Ca,L}), the transient outward current (I_o), the delayed rectifier K⁺ current (I_K), and the inward rectifier K⁺ current (I_K1) were decreased compared with those of F1β hamsters. Long-term treatment with the ET_A receptor antagonist significantly attenuated action potential duration prolongation and reduction of I_o, I_K, and I_{Ca,L} in BIO 14.6 ventricular cells. Long-term ET_A receptor blockade prevented the QT prolongation and ventricular arrhythmias and improved the survival rate in the cardiomyopathic hamsters.

**Conclusions**—Long-term treatment with an ET_A antagonist inhibits electrical remodeling such as downregulation of K⁺ and Ca²⁺ currents, action potential prolongation, and the increased QT interval and thereby suppresses ventricular arrhythmias in cardiomyopathic hearts. ET_A receptor blockade may provide a new strategy for the prevention of ventricular arrhythmias associated with heart failure. *(Circulation. 2002;106:613-619.)*

**Key Words:** endothelin  ■ ion channels  ■ cardiomyopathy  ■ electrophysiology

The prognosis of patients with congestive heart failure remains poor despite recent progress in medical therapy. It was assumed that ≈50% of affected patients die suddenly and unexpectedly. A major cause of sudden death in patients with heart failure appears to be ventricular tachyarrhythmias. Numerous studies that used cardiomyocytes of failing hearts have indicated that a variety of K⁺ currents are downregulated and the action potential duration is prolonged. Heterogeneous prolongation of the action potential can lead to the dispersion of refractoriness and the occurrence of ventricular reentrant arhythmias. However, medical therapy to prevent such electrophysiological alterations associated with heart failure has not been established.

It is well known that the sympathetic nervous and renin-angiotensin-aldosterone systems are activated in patients with heart failure. In addition, the endothelin (ET) system is also activated in various pathophysiological states including congestive heart failure. A number of studies have indicated that circulating endothelin-1 (ET-1) concentrations are elevated in congestive heart failure in both human and experimental animal models. It has been reported that long-term blockade of the endothelin-A (ET_A) receptor ameliorates ventricular contractile dysfunction in various animal models of congestive heart failure. More recently it has been found that long-term treatment with TA-0201, a selective ET_A antagonist, improved cardiac function and survival rate in cardiomyopathic Syrian hamsters. Since the BIO 14.6 cardiomyopathic hamsters were reported to have ventricular arrhythmias in the late stage, the ET_A receptor antagonist might prevent sudden cardiac death by inhibiting electrophysiological alterations associated with cardiomyopathic contractile dysfunction. Therefore, the present study was undertaken to examine the effect of long-term ET_A receptor blockade on the electrophysiological properties of ventricular...
cells, the surface ECG, and the survival rate in cardiomyopathic Syrian hamsters.

Methods

Experimental Animals
All experiments were performed according to the regulations of the Animal Research Committee of Chiba University Graduate School of Medicine and the Guide for the Care and Use of Laboratory Animals (NIH publication). Male cardiomyopathic hamsters (BIO 14.6) and normal F1β hamsters were purchased from Bio Breeders, Inc (Fitchburg, Mass) at the age of 17 to 19 weeks. These hamsters were randomized to receive no drug or TA-0201 (Tanabe Seiyaku Co), an orally active ETA receptor antagonist, from the age of 20 weeks. TA-0201 was mixed into their diet at a concentration of 30 ppm, and the daily dose of TA-0201 in the treated group was ~1.3 mg/kg per day.

Electrophysiology
Electrophysiological properties of ventricular cells were evaluated at the late heart failure stage in untreated (41 to 50 weeks) and TA-0201–treated BIO 14.6 hamsters (41 to 51 weeks) and in the age-matched F1β hamsters with (41 to 46 weeks) and without TA-0201 treatment (41 to 50 weeks). There were no significant differences in the age of the hamsters used for electrophysiological analysis among the groups. Single cells were isolated from the left ventricle free wall of the hamsters by conventional enzymatic digestion. Whole-cell membrane currents were recorded by the patch-clamp method as previously described.16 Current recordings were performed at 36.0°C with a glass patch pipette filled with (in mmol/L) potassium aspartate 110, KCl 20, MgCl2 1, potassium ATP 5, potassium phosphocreatine 5, EGTA 10, and HEPES-KOH buffer 5 (pH 7.4). The external solution contained (in mmol/L) NaCl 143, KCl 5.4, CaCl2 1.8, MgCl2 0.5, NaH2PO4 0.33, glucose 5.5, and HEPES-NaOH buffer 5 (pH 7.4). A liquid junction potential between the internal solution and the bath solution of 8 mV was corrected, and the series resistance was compensated by 50% to 70%.

Whole-cell membrane currents were recorded during 300-ms depolarizing or hyperpolarizing pulses from a holding potential of −80 mV at 0.1 Hz. The L-type Ca2+ current (I_{Ca,L}) was sensitive to 1 μmol/L nifedipine (Sigma), and the delayed rectifier K+ current (I_{K}) was measured. The transient outward current (I_{to}), which was sensitive to 4-aminopyridine (4-AP, 3 mmol/L, Wako), was measured by delivering 300-ms depolarizing pulses from a holding potential of −70 mV at 0.1 Hz in a solution containing 2 mmol/L CoCl2. The Na+ current was inactivated by a 10-ms prepulse to −40 mV. Current-clamp experiments were also conducted in the whole-cell recording mode at 36.0°C. The cells were stimulated by rectangular 2-ms currents (10 nA) through the pipette at 0.2 Hz. The cells used for measurements of membrane capacitance, current density, and action potentials were chosen at random. The data were summarized from cells isolated from at least 5 animals in each group. It was confirmed that TA-0201 at concentrations up to 1 μmol/L, which was 10 times higher than the in vivo concentrations attainable with therapeutic dose, did not produce any appreciable effect on the membrane currents.

ECG Telemetry and Survival Rate
The surface ECG was recorded from unrestrained conscious hamsters with the use of a telemetry system at the age of 40 weeks. The animals were anesthetized with sodium thiopental (30 to 50 mg/kg IP), and a dorsal incision was performed. A small transmitter (TA 10EA-F20, Data Science International, Inc) with 2 coiled wires, which were subcutaneously pulled to the right shoulder and left flank regions, was placed in the dorsal cavity. All hamsters were allowed to recover for 3 days after the operation. A caged hamster was placed on a receiver board (RA1310, Data Science International, Inc) and the ECG was recorded for 10 minutes between 2 and 4 AM. The ECG signals were stored on a DVD-ROM disk and analyzed by a software package (Fuklet system, Dainippon Pharmaceutical Co Ltd).

In a separate series of experiments, effects of long-term ETA receptor blockade on survival were examined in BIO 14.6 cardiomyopathic hamsters. From 20 weeks of age, TA-0201 was administered in 15 cardiomyopathic hamsters until 55 weeks of age. The survival rate was compared with that of 15 untreated cardiomyopathic hamsters.

Statistics
All values are presented as mean±SEM. Student’s t test or ANOVA was used for statistical analysis. Comparison of survival rate was made by a log-rank test with the Kaplan-Meier method. Differences were considered significant at the level of P<0.05.

Results

Cell Capacitance and Action Potential
Membrane capacitance (Cm) was used as an index of cell size. Cm values for left ventricular myocytes of F1β and BIO 14.6 hamsters were 197±10 pF (80 cells from 18 F1β hamsters) and 215±10 pF (82 cells from 18 BIO 14.6 hamsters), respectively. The average Cm of BIO 14.6 myocytes was slightly but significantly greater than that of F1β cells (P<0.05). However, the Cm values of the ventricular cells of BIO 14.6 and F1β hamsters treated with TA-0201 from 20 weeks of age were 195±8 pF (85 cells from 18 hamsters) and 203±9 pF (40 cells from 9 hamsters), respectively, which were not significantly different from that of untreated F1β hamsters.

Representative action potentials recorded from left ventricular myocytes are shown in Figure 1A. Action potential durations at 0 mV (APD0) and −60 mV (APD60) levels of untreated BIO 14.6 myocytes were significantly longer than those of F1β myocytes. However, APDs of ventricular myocytes of BIO 14.6 hamsters treated with TA-0201 were not significantly different from untreated or drug-treated F1β hamsters (Figure 1B). There were no significant differences in other action potential parameters such as resting membrane potential and action potential amplitude among 4 groups.

Figure 2 shows scatterplots of actual APD values in 4 groups. Dispersion of APDs in ventricular cells of untreated BIO 14.6 hamsters was greater than those of untreated F1β and TA-0201–treated F1β and BIO 14.6 hamsters. The standard deviations of APD-60 values in untreated F1β and BIO 14.6 and TA-0201–treated F1β and BIO 14.6 ventricular cells were 8, 47, 8, and 13 ms, respectively. Thus, APDs in cardiomyopathic ventricular cells were prolonged and dispersed, which was inhibited by long-term treatment with TA-0201.

Density of Membrane Currents
To define the ionic mechanism(s) responsible for the APD prolongation in cardiomyopathic ventricular cells, membrane currents were measured with patch-clamp techniques in left ventricular cells isolated from 4 groups of hamsters. Representative current traces of I_{to}, a major repolarizing current in hamster ventricular cells, are depicted in Figure 3A. The density of I_{to} in BIO 14.6 ventricular cells was significantly lower than that in F1β ventricular cells. Although long-term treatment with TA-0201 hardly affected the density of I_{to} in F1β ventricular cells, it maintained the density of I_{to} in
ventricular cells of BIO 14.6 hamsters to a level comparable to that in normal hamster cells.

The density of the inward rectifier K⁺ current (Iₖ) was also measured in left ventricular cells of 4 groups. Representative records of Iₖ elicited by hyperpolarizing test pulses from a holding potential of −40 mV are depicted in Figure 4A, and the current density-voltage relations for the late current in untreated F1β and BIO 14.6 cells are summarized in Figure 4B. The densities of both the outward (at −50 mV) and inward components (at −100 mV) of Iₖ in untreated BIO 14.6 cells were significantly lower than those in normal F1β cells (Figure 4C). Treatment with TA-0201 failed to improve the density of Iₖ in BIO 14.6 hamsters significantly.

As shown in Figure 5, the inward current, elicited by depolarizing pulses from a holding potential of −40 mV, was recorded from BIO 14.6 ventricular cells and compared with that in F1β cells. The density of the nifedipine-sensitive Iₖ,L at 0 mV in BIO 14.6 ventricular cells was significantly smaller than that in normal F1β cells, and that in TA-0201–treated BIO 14.6 ventricular cells was slightly but significantly greater than that in untreated BIO 14.6 cells (Figure 5C). The density of Iₖ, measured as a tail current after a depolarization pulse to +10 mV, in BIO 14.6 cells was significantly smaller than that in F1β cells. Long-term treatment with TA-0201 significantly inhibited the decrease of Iₖ in BIO 14.6 hamsters (Figure 5D).

**ECG and Survival Rate**

The surface ECG was recorded from 3 groups of hamsters. There were no significant differences in the heart rate among 3 groups. The QT interval in BIO 14.6 hamsters (124 ± 5 ms) but not that in TA-0201–treated BIO 14.6 hamsters (109 ± 4 ms) was significantly longer than that in control F1β hamsters (111 ± 2 ms) (Figure 6B).

Premature ventricular contractions (PVCs) were observed in both BIO 14.6 and F1β hamsters at 40 weeks. However, the percentage of PVCs in the total heart rate and the number of PVCs in untreated BIO 14.6 hamsters were significantly greater than those in normal hamsters (Figure 6, A, C, and D). TA-0201 significantly decreased the percentage and the absolute number of PVCs.

Long-term treatment with TA-0201 from the age of 20 weeks significantly improved the survival rate of the BIO 14.6 cardiomyopathic hamsters (Figure 7). At 40 weeks, the survival rate of cardiomyopathic hamsters was 73% in the untreated group and 93% in the TA-0201–treated group.

**Discussion**

The BIO 14.6 Syrian hamster is an animal model of genetically determined cardiomyopathy, in which cardiac myolysis...
develops until 10 weeks of age and hypertrophy develops at 10 to 20 weeks of age before overt congestive heart failure, and severe ventricular arrhythmias and sudden cardiac death are often encountered in this strain. Numerous studies have indicated that electrophysiological remodeling such as changes of action potential, \( K^+ \) and \( Ca^{2+} \) currents occurs in hypertrophy and heart failure (see review by Tomaselli and Marbán). Thuringer et al reported that the \( I_{\text{to}} \) and \( I_{\text{Ca,L}} \) densities were decreased and the APD was prolonged in ventricular cells isolated from MS 200 Syrian hamsters in which dilated cardiomyopathy develops without any detectable hypertrophic phase. In this study, the densities of \( I_{\text{to}} \), \( I_{\text{K1}} \), \( I_{\text{K}} \), and \( I_{\text{Ca,L}} \) were decreased as well in BIO 14.6 strain. Similar reduction of \( I_{\text{to}} \) and \( I_{\text{K1}} \) has been observed in ventricular cells isolated from failing human hearts. In terms of \( I_{\text{Ca,L}} \), various changes such as an increase, no change, and a decrease in the density of \( I_{\text{Ca,L}} \) have been reported in myocytes of failing human hearts. In BIO 14.6 hamsters, the density of \( I_{\text{Ca,L}} \) was significantly depressed at the late heart failure stage, although it was unaltered at 20 weeks (unpublished observations). The dihydropyridine binding sites in cardiac membrane preparations were reportedly increased in early hypertrophic phase but decreased in the late decompen-sated stage of BIO 14.6 cardiomyopathic hamsters. Therefore, it is possible that the change in the \( I_{\text{Ca,L}} \) density may be dependent on the stage of the cardiomyopathy.

Figure 3. Transient outward current (\( I_{\text{to}} \)) recorded from ventricular cells of untreated and TA-0201–treated F1β and BIO 14.6 hamsters. A, Representative current traces recorded from untreated F1β, BIO 14.6, and TA-0201–treated BIO 14.6 cells; membrane capacitance of each cell is indicated at upper part of the record. B, Summarized data of current density-voltage relations for 4-aminopyridine (4-AP)-sensitive peak current. Data represent mean±SEM of 14 to 16 cells from 5 animals for each group. *\( P<0.05 \) vs control (untreated F1β cells). #\( P<0.05 \) vs untreated BIO 14.6.

Figure 4. Inward rectifier \( K^+ \) current (\( I_{\text{K1}} \)) recorded from ventricular cells of untreated and TA-0201–treated F1β and BIO 14.6 hamsters. A, Representative current traces recorded from untreated F1β, BIO 14.6, and TA-0201–treated BIO 14.6 cells; membrane capacitance of each cell is indicated at upper part of the record. B, Graph shows current density-voltage relations for current measured at end of test pulses. Data represent mean±SEM of 19 to 21 cells from 6 animals. *\( P<0.05 \) vs control. C, Summa-rized data of density of \( I_{\text{K1}} \) at −50 mV and −100 mV.
species including human and cardiomyopathic hamsters. In this study, APDs in untreated BIO 14.6 myocytes were significantly longer than those in normal F1β myocytes. A decrease in $I_{Ca,L}$ is expected to shorten APD, whereas a decrease in $I_K$, $I_{K1}$ is expected to prolong APD. Therefore, reduction of outward K+ currents might mainly contribute to the prolongation of APD. Increased dispersion of APD might lead to inhomogeneity of effective refractory period and QT interval. The QT interval in surface ECG was increased in BIO 14.6 hamsters, as observed in human and animal models with heart failure.22,23 Such electrophysiological abnormalities might provide the substrate for reentrant ventricular arrhythmias. The density of the Na+/Ca2+ exchange current was greater in BIO 14.6 cells than in F1β cells and the current increase was inhibited by long-term treatment with TA-0201, although these differences were statistically insignificant (Matsumoto et al, unpublished observations). Reduction of repolarizing K+ current may also increase the susceptibility to arrhythmias arising from accelerated phase 4 diastolic depolarization and afterdepolarization-mediated triggered arrhythmias.

It is well acknowledged that plasma levels of ET-1 are increased in patients with heart failure without respect to the underlying cause. Although the precise pathophysiological role of ET-1 in heart failure remains uncertain, it has been suggested that ET-1 is secreted from cardiomyocytes or nonmyocytes and acts as a local autocrine or paracrine hormone to produce vasoconstriction, positive inotropy, and

Figure 5. L-type Ca2+ current ($I_{Ca,L}$) and delayed rectifier K+ current ($I_K$) recorded from ventricular cells of untreated and TA-0201-treated F1β and BIO 14.6 hamsters. A, Representative current traces; membrane capacitance of each cell is indicated at upper part of the record. B, Graph shows current density-voltage relations for current measured at peak of $I_{Ca,L}$. Data represent mean±SEM of 19 to 21 cells from 6 animals. *P<0.05 vs control (untreated F1β cells). C, Summarized data of nifedipine-sensitive current at 0 mV. D, Densities of tail current of $I_K$ after depolarization pulse to +10 mV.

Figure 6. Effects of TA-0201 on QT interval and ventricular arrhythmias in cardiomyopathic hamsters. A, Ventricular arrhythmias observed in an untreated BIO 14.6 hamster; B, QT interval; C, percentage of PVCs in total heart rate; and D, absolute number of PVCs per 10 minutes. Data represent mean±SEM of 9 to 10 hamsters.
hypertrophy.\textsuperscript{12,24,25} There are several studies showing beneficial effects of the ET receptor blockade on survival, hemodynamic parameters, and histological remodeling.\textsuperscript{11–13} This study has demonstrated for the first time that long-term treatment with an ETA receptor antagonist prevents electrical remodeling such as reduction of $I_{Na}$, $I_{Ca}$, and $I_{Ca,L}$, and prolongation of APD in ventricular cells and improves QT prolongation in the ECG of cardiomyopathic hamsters. Beneficial effects of the ETA antagonist TA-0201 on survival rate might be partly ascribed to the inhibition of ventricular arrhythmias by lessening electrical inhomogeneity resulting from downregulation of ion channels.

The mechanism(s) by which the ETA receptor antagonist inhibits electrical remodeling in cardiomyopathic hearts remain unclear. The electrical remodeling might be caused by cell hypertrophy. In this study, membrane capacitance, an index of cell size, was slightly increased in untreated BIO 14.6 ventricular cells but not in TA-0201–treated cells. However, an increase in cardiac cell size is not always observed in other experimental models of heart failure.\textsuperscript{4,5} Although downregulation of K$^+$ channels is commonly observed.

Improvement of cardiac function with the ETA antagonist might inhibit the electrical remodeling indirectly, although the relation between mechanical function and ion channel remodeling has not been established. Indeed, long-term treatment with TA-0201 was reported to improve hemodynamic parameters markedly in BIO 14.6 cardiomyopathic hamsters.\textsuperscript{11}

More recently it has been reported that long-term treatment with an ETA antagonist normalizes the altered expression levels of mRNA encoding cardiac molecular markers such atrial natriuretic peptide, sarcoplasmic reticulum Ca$^{2+}$-ATPase, and ryanodine receptors in failing rat hearts.\textsuperscript{26} Therefore, the blockade of ETA receptors might modulate the expression levels of cardiac ion channels through some intracellular mechanisms. However, the intracellular mechanism through which ETA receptor antagonists prevent electrical remodeling in the failing hearts remains unclear.

The present study was mainly focused on the remodeling of repolarizing ion currents and APD in cardiomyopathic hearts. They are important for the induction of abnormal automaticity and reentrant arrhythmias. The prevention of ionic remodeling may be an upstream approach to antiarrhythmic therapy.\textsuperscript{27} However, intramyocardial conduction is also important for the establishment of reentrant pathway. Changes of the fast Na$^+$ current and gap junctional function might occur in cardiomyopathic hearts. Therefore, it may be another issue to be answered whether long-term ETA receptor blockade influences the myocardial conduction properties in failing hearts.

In conclusion, long-term treatment with an ETA antagonist inhibits electrical remodeling such as downregulation of K$^+$ and Ca$^{2+}$ currents, action potential prolongation, and the increased QT interval and improves the survival rate. ETA receptor blockade may provide a new strategy for the prevention of ventricular arrhythmias associated with heart failure.

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**References**


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