Selective Endothelin ET\textsubscript{B} Receptor Antagonist Improves Left Ventricular Function but Exaggerates Degeneration of Cardiomyocytes in J2N-k Hamsters

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Background Endothelin-1 (ET-1) receptor antagonist is expected to improve the prognosis of patients with heart failure, but the role of the ET\textsubscript{B} receptor in cardiac function and structure is complicated. In the present study the NADPH diaphorase activity and ET-1 content in the failing heart treated with ETA or ETB receptor antagonist were evaluated in a model of dilated cardiomyopathy.

Methods and Results Selective ETA receptor antagonist, ABT-627 (10 mg/kg per day), or selective ETB antagonist, A-192621 (30 mg/kg per day), was given to 22-week-old J2N-k cardiomyopathic hamsters for 8 weeks. The effects of ABT-627 and A-192621 on cardiac function, left ventricular (LV) histology, ET-1 content and NADPH diaphorase activity in the LV were evaluated. Treatment with ABT-627, but not A-192621, significantly decreased ET-1 content and NADPH diaphorase activity. Although the improvement of LV function was modest, ABT-627 prevented tissue damage in J2N-k hamsters. In contrast, A-192621 worsened the degeneration of cardiomyocytes despite improving hemodynamic parameters.

Conclusions Selective ETA antagonist, but not ETB antagonist, reduced the ET-1 content as well as the NADPH diaphorase activity, and preserved the fine structure of LV myocardium in cardiomyopathic hamsters. Long-term blockade of ETB receptor might worsen the degeneration of cardiomyocytes through the ET-1/ETA system even if LV function could be improved. (Circ J 2005; 69: 107–113)

Key Words: Cardiomyopathy; Endothelin-1; ETs receptor antagonist; Histology; NADPH diaphorase activity

Endothelin (ET)-1 plays an important role in the progression of congestive heart failure (CHF). It is known that plasma ET-1 concentrations are elevated in patients with CHF and correlate with severity.\textsuperscript{1,2} ET-1 may act as a poten growth factor inducing cardiac hypertrophy\textsuperscript{3} and exert positive inotropic and chronotropic effects on the heart.\textsuperscript{4,5} Moreover, extremely elevated concentrations of ET-1 might be toxic and cause local myocyte damage, leading to cardiac dysfunction.\textsuperscript{6} Although both ETA receptor-selective antagonist and nonselective ET\textsubscript{A}/ET\textsubscript{B} receptor antagonist have been reported to improve cardiac function in CHF, it is still controversial whether selective ETA receptor blockade or nonselective ET\textsubscript{A}/ET\textsubscript{B} receptor blockade is preferable for the treatment of patients with CHF, because the pathophysiological role of ET\textsubscript{A} receptors in CHF has not been fully elucidated.\textsuperscript{7–10} Furthermore, little is known about the effect of long-term treatment with ET\textsubscript{A} receptor blockade on cardiac function and structure in CHF.

The vast majority of cases of CHF are caused by heart muscle disease (cardiomyopathy). Within the WHO categorization of cardiomyopathy, the most common cause of heart failure is dilated cardiomyopathy, defined as a ventricular chamber exhibiting increased diastolic and systolic volumes and a low ejection fraction.\textsuperscript{11} Idiopathic dilated cardiomyopathy (IDC) is diagnosed by excluding significant coronary artery disease, valvular abnormalities, and other causes. Histological features are nonspecific and consist of myocardial cell hypertrophy as well as atrophy and varying amounts of increased interstitial fibrosis.\textsuperscript{12} Recently, activation of the ET-1 system with increased tissue ET-1 content in the myocardium of IDC patients has been reported,\textsuperscript{13–15} but whether ET receptor-mediated ET-1 actions are involved in the progression of CHF resulting from IDC remains to be determined.

Recent studies have shown that the expression of inducible nitric oxide synthase (iNOS), as well as that of ET-1, in the myocardium is elevated in patients with IDC.\textsuperscript{16,17} Several neurohumoral factors activated in chronic heart failure might augment cardiac iNOS expression and could cause cardiac dysfunction.\textsuperscript{18,19} There might be some relation between ET-1 and iNOS in the failing heart but the precise mechanisms and the role of ET-1 and iNOS in CHF caused by IDC have not been clarified. The aim of this study was to compare the chronic effect of selective ETA and ETB receptor antagonist on cardiac function, ET-1 content, ultrastructure and histochemical changes of reduced nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase.
activity in J2N-k hamsters, a model of IDC.

Methods

Animals

The study animals were J2N-k cardiomyopathic hamsters and J2N-n normal control hamsters (SLC, Inc, Hamamatsu, Japan). All animals were kept in a specific pathogen-free facility under controlled conditions of temperature (20–24°C) and humidity (40–70%), with a 12-h lighting cycle, and were given free access to standard laboratory rat chow (MF, Oriental Yeast, Tokyo, Japan) and tap water. This investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and all procedures were in accordance with institutional guidelines for animal research.

Experimental Protocol

Male 22-week-old J2N-k cardiomyopathic hamsters were randomly divided into 3 groups and given ABT-627 (a selective ETα receptor antagonist, n=9), A-192621 (a selective ETβ receptor antagonist, n=10), or vehicle (n=11) for 8 weeks. Age- and sex-matched J2N-n hamsters were used as normal controls (n=10). ABT-627 (10 mg/kg per day), A-192621 (30 mg/kg per day), or vehicle was given twice daily by gavage. The doses of ABT-627 and A-192621 used in this study have previously been shown to almost abolish the exogenous ET-1 induced pressor effect and sarafotoxin S6c-induced depressor and pressor effects, respectively. Eight weeks after treatment, each hamster was anesthetized with sodium thiobarbital (Inactin, 100 mg/kg) and placed on a surgical tray that maintained rectal temperature between 37°C and 38°C.

Echocardiography was performed using a commercially available ecocardiographic system equipped with a 12-MHz phased-array transducer (SONOS 5500, Phillips, The Netherlands). End-diastolic and systolic dimensions, E/A ratio in transmitral flow, and E wave deceleration rate (EDR) were measured. Ejection fraction (EF) was calculated by modifying Simpson’s method, which uses 2- and 3-chamber views. After tracheotomy, the right external carotid artery was catheterized with polyethylene tubing (SP-10, Natume, Tokyo, Japan) filled with heparinized saline for measurement of arterial blood pressure. After stable mean arterial pressure and heart rate were obtained, a 23-gauge needle connected to a heparinized saline-filled catheter was inserted through the left ventricular (LV) apex under closed-chest conditions for measuring LV pressure. Mean arterial and LV pressures were continuously recorded on a polygraph (RM6000, Nihon Koden, Tokyo, Japan). All data were analyzed by averaging 10 consecutive beats. After hemodynamic measurement of blood pressure, the heart and lungs were excised under anesthesia for morphological study, immunohistochemistry and measurement of ET-1 content.

Light and Electron Microscopy Studies

For the light microscopic study, the specimens were fixed in 10% formaldehyde, embedded in paraffin, and cut into 4-mm-thick sections, which were stained with hematoxylin–eosin and Mallory–azan. To evaluate the mean diameter of LV cardiomyocytes, the shortest diameter of each cardiomyocyte was measured only in nucleated transverse sections; 150 cardiomyocytes in each LV were measured using an ocular micrometer disc with a linear scale at a magnification of ×400, and the average cardiomyocyte diameter of each specimen was calculated.

For the semi-quantitative analysis of the histological changes, each finding of (a) disarray of myofibers, (b) hypertrophy of myofibers, (c) scarcity of myofibrils, (d) nuclear changes and (e) vacuolization was graded by a previously published method: grade (−) signifies no apparent change; 1+, minimal degree; 2+, moderate degree; 3+, marked degree; and 4+, excessive degree. The sum of the graded scores in each sample was defined as the semi-quantitative score.

For the transmission electron microscopic study, the specimens were fixed in 4% paraformaldehyde containing 0.25% glutaraldehyde and 4.5% sucrose, postfixed in 1% osmium tetroxide, dehydrated through a graded series of ethanol, and embedded in Epon. Ultrathin sections obtained from the embedded blocks were stained with uranyl acetate and lead citrate, and were examined with a Hitachi H-7000 electron microscope.

Immunohistochemistry for Endothelin-1

For the immunohistochemical light microscopic studies, additional sections were obtained from the paraffin block. The sections were incubated overnight at 4°C with monoclonal antibody against endothelin-1 (#CP44; Calbiochem, EMD Biosciences, Inc, Germany). After incubation with secondary antibody, the sections were allowed to react with Vectastain Elite ABC reagent (Vector Laboratories, Burlingame, CA, USA), and then reacted with the peroxidase-dase substrate solution (Vectastain 3’3’-diaminobenzidine substrate kit; Vector Laboratories). Tissue Endothelin-1 Assay

ET-1 was extracted from the LV as described elsewhere. Briefly, the tissues were weighed and homogenized for 60 s in 4 ml of ice-cold organic solution (chloroform/methanol, 2:1, including 1 mmol/L N-ethylmaleimide). The homogenates were left overnight at 4°C and then 0.4 ml of 0.09% trifluroacetic acid (TFA) was added to the homogenates. Homogenates were centrifuged at 3,000 rpm for 30 min and the supernatant was stored. Aliquots of supernatant were diluted 1:10 with a 0.09% TFA solution and applied to Sep-Pak C18 cartridges. Each sample was eluted with 3 ml of 63.3% acetonitrile and 0.1% TFA, then dried in a centrifugal concentrator, and the dried residue was reconstituted in assay buffer for radioimmunoassay (RIA) of the clear solution. The recovery of ET-1 was approximately 80%. RIA for tissue ET-1 was performed as described elsewhere, using ET-1 antiserum which does not cross-react with big ET-1 and can distinguish between ET (1-21) and the C-terminally extended form, ET (1-39).

NADPH Diaphorase Histochemistry

The NADPH diaphorase histochemistry was performed by a method reported previously. The hearts were snap frozen and embedded in Tissue-Tek OCT compound (Sakura Finetechanical, Tokyo, Japan). Frozen sections, at 20-μm-thickness, were cut using a cryostat and fixed with 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) for 1 h at room temperature. After fixation, the sections were rinsed with phosphate-buffered saline, and incubated for 1 h at 37°C in the dark with reaction solution consisting of phosphate buffer (pH 7.4) containing 0.1% NADPH (Sigma-Aldrich Fine Chemicals, MO, USA) and 0.02% nitroblue tetrazolium (Sigma). A negative control was made.
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Results

J2N-k Hamsters Before Treatment

The 22-week-old J2N-k cardiomyopathic hamsters, compared with J2N-n hamsters, showed a significant decrease in mean arterial pressure (100±6 vs 137±6 mmHg) and LV peak negative dP/dt (4,067±233 vs 9,250±608 mmHg/s, p<0.05). LV end-diastolic pressure (J2N-k: 7.3±3.0 vs J2N-n: 2.4±0.7 mmHg) tended to increase and peak positive dP/dt (J2N-k: 6,872±818 vs J2N-n: 8,360±658 mmHg/s) tended to decrease. The hearts from the J2N-k hamsters weighed the same as those from the J2N-n hamsters. Mild degeneration of the cardiomyocytes and interstitial fibrosis were observed in the LV myocardium of 22-week-old J2N-k hamsters (Fig 1).

Tissue Weights and Hemodynamic Measurements

The ratios of left and right ventricular weights to body weight (LVW/BW and RVW/BW) tended to increase in the vehicle-treated J2N-k hamsters compared with the J2N-n control hamsters. Although there not any statistical significance, ABT-627 but not A-192621 treatment tended to suppress the increase in LVW/BW (Table 1).

Regarding hemodynamics, vehicle-treated J2N-k hamsters showed a significant decrease in mean blood pressure, LV peak positive and negative dP/dt, and EF, and an increase in LV end-diastolic pressure, E/A ratio and ED rate (Table 1, Fig 2). Chronic treatment with ABT-627 in the J2N-k hamsters improved the E/A ratio and ED rate, but had no effect on heart rate, mean blood pressure, LV end-diastolic pressure, LV peak positive and negative dP/dt or EF. Chronic treatment with A-192621 significantly increased heart rate, mean blood pressure, LV peak positive and negative dP/dt and EF, and improved E/A ratio and ED rate similarly to ABT-627 (Table 1, Fig 2).

Light and Electron Microscopy Studies

Vehicle-treated 30-week-old J2N-k hamsters showed

Table 1 Effects of ABT-627 and A-192621 Treatment on J2N-k Cardiomyopathic Hamsters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>J2N-k</th>
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<tbody>
<tr>
<td></td>
<td>J2N-n (n=10)</td>
<td>J2N-k (n=11)</td>
</tr>
<tr>
<td></td>
<td>J2N-n (n=9)</td>
<td>J2N-k (n=10)</td>
</tr>
<tr>
<td>BW (g)</td>
<td>120±3</td>
<td>122±2</td>
</tr>
<tr>
<td>LVW/BW (mg/g)</td>
<td>1.97±0.01</td>
<td>2.05±0.04</td>
</tr>
<tr>
<td>RVW/BW (mg/g)</td>
<td>0.47±0.02</td>
<td>0.58±0.03</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>392±9</td>
<td>364±18</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>140±5</td>
<td>91±4*</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>2.4±0.5</td>
<td>20.2±1.6*</td>
</tr>
<tr>
<td>max dP/dt (mmHg/s)</td>
<td>7.54±6.9</td>
<td>4.95±3.74</td>
</tr>
<tr>
<td>min dP/dt (mmHg/s)</td>
<td>7.57±9.33</td>
<td>3.08±2.71*</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*p<0.05 vs J2N-n control hamsters, *p<0.05 vs J2N-k hamsters treated with vehicle.

BW, body weight; LVW, left ventricular weight; RVW, right ventricular weight; HR, heart rate; MBP, mean blood pressure; LVEDP, LV end-diastolic pressure; max and min dP/dt, peak positive and negative dP/dt.

Statistical Analysis

Values are means±SEM. Statistical analysis was performed by one-way ANOVA. When significant differences were identified, the Tukey multiple comparison test was applied to determine the level of significance. For all comparisons, differences were considered significant when p<0.05.

Fig 1. Macroscopic (Upper panel) and light microscopic (Lower panel) photographs (H&E; ×100). Samples were obtained from 22-week-old J2N-n (A, D) and J2N-k (B, E), and 30-week-old J2N-k hamsters (C, F). In the hearts of 22-week-old J2N-k hamsters, a slightly dilated LV cavity, mild degeneration of cardiomyocytes and interstitial fibrosis are observed (B, E). In the 30-week-old hamsters, marked dilatation of the LV cavity is seen (C). In the most degenerated region, hypertrophied cardiomyocytes and increased interstitial fibrosis are observed (F).

by omission of △NADPH, and no staining was observed.

Light and Electron Microscopy Studies

Vehicle-treated 30-week-old J2N-k hamsters showed
Fig 2. Echocardiography. Representative pulsed-wave Doppler spectra of mitral inflow from 30-week-old J2N-N and J2N-k hamsters are shown. Increased E/A ratio and increased E wave deceleration rate (ED rate, cm/s²) in the J2N-k hamsters were improved by each treatment, but the decreased ejection fraction (EF) was improved only by A-192621. Each column and bar represent the mean ± SEM. *p<0.05 compared with J2N-n control hamsters. #p<0.05 compared with vehicle-treated J2N-k cardiomyopathic hamsters.

Fig 3. Light micrographs. (Upper panel: A–D) hematoxylin-eosin (×100). (Middle panel: E–H; ×100): immunohistochemistry for endothelin (ET)-1. (Lower panel: J–M; ×250): NADPH diaphorase histochemistry. Cardiomyocytes of the LV myocardium of J2N-n hamsters show normal configuration and no reactivity for ET-1 or nitric oxide synthase (A, E, J). Hypertrophy of cardiomyocytes and interstitial fibrosis are seen in J2N-k hamsters (B). Increased ET-1 and NADPH diaphorase activity in cardiomyocytes are also observed (F, K). Chronic treatment with ABT-627 almost completely suppressed the hypertrophy of cardiomyocytes, and decreased ET-1 and NADPH diaphorase activities in the cardiomyocytes (C, G, L). Treatment with A-192621 worsened the degeneration of cardiomyocytes and increased interstitial fibrosis in the LV myocardium of J2N-k cardiomyopathic hamsters, and had a very small effect on ET-1 and NADPH diaphorase activities (D, H, M).
severe LV dilatation (Fig 1C). Marked hypertrophy of cardiomyocytes (mean diameter: 26.1±0.6 vs J2N-n; 17.6±0.1 mm, p<0.05) and interstitial fibrosis were also observed (Figs 1F,3B). Under electron microscopy, bizarrely shaped nuclei and myofibrillar lysis were found in J2N-k hamsters (Fig 4B). Chronic treatment with ABT-627 almost completely suppressed the hypertrophy of cardiomyocytes (18.4±0.5 mm) and preserved the fine structure of the LV myocardium (Fig 4C), whereas chronic treatment with A-192621 had no effect on the diameter of cardiomyocytes (27.3±0.7 mm) and exaggerated all features of degeneration in the LV myocardium (Figs 3D,4D).

The semi-quantitative analysis clarified the histological changes after each treatment: A-192621 increased the scores of hypertrophy and nuclear changes in the cardiomyocytes, and increased the proliferation of collagen fibers in the interstitium (Table 2).

### Immunohistochemical Localization of Endothelin-1

ET-1 staining in the LV tissues of vehicle-treated J2N-k hamsters was much stronger than that in J2N-n control hamsters. The expression of ET-1 was localized to cardiomyocytes, not interstitial cells (Fig 3F). In the tissues of J2N-k hamsters treated with ABT-627, the expression of ET-1 was attenuated, but there was no change in the tissues of the A-192621-treatment group (Fig 3G,H).

### Endothelin-1 Content in the LV Myocardium

The ET-1 content in the LV of J2N-k cardiomyopathic hamsters was approximately 10-fold higher than in the LV of J2N-n normal hamsters at 30 weeks of age. The concentration of ET-1 peptide in J2N-k hamsters decreased by 40% in J2N-k hamsters treated with ABT-627. However, A-192621 did not affect the ET-1 concentrations in the LV of J2N-k hamsters (Fig 5).

### NADPH Diaphorase Activity

The NADPH diaphorase activity was not found in the cardiomyocytes of the J2N-n control hamsters (Fig 3J). Increased activity, which might be caused by iNOS, was observed in the LV cardiomyocytes of the J2N-k hamsters (Fig 3K). The increase in NADPH diaphorase activity was efficiently suppressed by treatment with ABT-627 (Fig 3L).

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### Table 2 Semiquantitative Score of the Histological Changes After Treatment With ABT-627 or A-192621

<table>
<thead>
<tr>
<th>Histological findings</th>
<th>Control J2N-n</th>
<th>J2N-k Vehicle</th>
<th>J2N-k ABT-627</th>
<th>J2N-k A-192621</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disarray of myofibers</td>
<td>(-)</td>
<td>1.2±0.1</td>
<td>1.1±0.1</td>
<td>1.5±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertrophy of myofibers</td>
<td>(-)</td>
<td>2.6±0.2</td>
<td>1.9±0.1</td>
<td>3.0±0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Scarcity of myofibrils</td>
<td>(-)</td>
<td>1.7±0.1</td>
<td>1.6±0.1</td>
<td>1.6±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Nuclear changes</td>
<td>(-)</td>
<td>1.7±0.1</td>
<td>1.5±0.1</td>
<td>2.4±0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Vacuolization</td>
<td>(-)</td>
<td>1.1±0.1</td>
<td>1.2±0.1</td>
<td>1.6±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Proliferation of collagen fibers</td>
<td>(-)</td>
<td>2.0±0.2</td>
<td>1.8±0.1</td>
<td>2.7±0.2</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

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Fig 4. Electron micrographs. The cardiomyocytes from J2N-n hamsters have a normal ultrastructure (A). In J2N-k hamsters, bizarrely shaped nuclei and myofibrillar lysis are found (B). Chronic treatment with ABT-627 preserved the fine the structure of the LV myocardium (C). In contrast, chronic treatment with A-192621 exaggerated the degeneration observed in the LV myocardium (D). Scale bar=1 μm.

Fig 5. Endothelin (ET)-1 content in the LV myocardium. The increased ET-1 content in the LV of J2N-k cardiomyopathic hamsters was suppressed by 40% by ABT-627 treatment. Each column and bar represent the mean±SEM. *p<0.05 compared with J2N-n control hamsters. #p<0.05 compared with vehicle-treated J2N-k cardiomyopathic hamsters.
Discussion

This is the first study to examine the effect of chronic ETs receptor blockade in an animal model of cardiomyopathy. J2N-k cardiomyopathic hamsters were established through consecutive crossbreeding between healthy Golden hamsters and BIO 14.6 cardiomyopathic hamsters. In the present study, 22-week-old J2N-k hamsters showed moderate cardiac dysfunction, degeneration, but not hypertrophy, of cardiomyocytes and interstitial fibrosis, which are findings that are consistent with previous reports. J2N-k hamsters at 30 weeks of age, however, exhibited severe cardiac dysfunction and marked dilation of the ventricular cavity. Histologically, hypertrophy of cardiomyocytes, with varying degrees of degeneration, and increased interstitial fibrosis were observed. These hemodynamic and morphological findings are also found in human patients with IDC. Thus, the J2N-k hamster might be a model for human IDC, in addition to BIO 14.6 hamsters, which are considered to be a model of hypertrophic cardiomyopathy.

In the present study, we investigated the content and localization of ET-1 in the failing heart of J2N-k hamsters. The ET-1 content in the LV of J2N-k hamsters was markedly higher than that of J2N-n hamsters (Fig 5) and the enhanced expression of ET-1 peptide was localized to the cardiomyocytes, not the interstitial cells (Fig SF). These findings are compatible with the results of previous studies using either the myocardium of patients with cardiomyopathy or animal models of cardiomyopathy.

Chronic treatment with ABT-627, a selective ETA antagonist, completely prevented the increase in LV tissue weight and myocyte hypertrophy in J2N-k hamsters, although the beneficial effects on cardiac function were modest in terms of the improvement of diastolic function. Recently, Yamauchi-Kohno et al reported that chronic treatment with an ETA receptor antagonist (TA-0201) significantly reduced the progression of cardiac dysfunction in BIO 14.6 hamsters at a terminal stage of CHF, besides suppressing hypertrophy of cardiomyocytes. The reason for this difference is unknown, but the efficacy of the ETA receptor antagonist might depend on the type of cardiomyopathy and/or the timing and duration of medication. However, it is apparent that the ET-1/ETA system has an important role in the hypertrophy of cardiomyocytes in both hypertrophic and dilated cardiomyopathies.

In contrast, chronic treatment with A-192621, a selective ETs antagonist, significantly increased the heart rate, blood pressure, LV peak positive dp/dt and EF, and decreased the E/A ratio and ED rate (Table 1, Fig 2). Some of these hemodynamic parameters are load-dependent, and the increased dp/dt of the LV pressures might be simply related to increased blood pressure and heart rate. Although ET-Induced afterload changes might affect the hemodynamics, the improvements in EF, E/A ratio and ED rate were significant. ETs receptors in cardiac interstitial cells act in the local clearance of ET-1 peptide. When the ETs receptor is blocked, enhanced ETA-mediated vasoconstriction has been observed, because ETs receptors in the vascular beds play a role in vasodilator effects and the clearance of ET-1. Although the ET-1 content in the LV myocardium showed no significant change after treatment with A-192621 compared with vehicle, chronic blockade of ETs receptors might increase ETA-mediated the positive inotropic and chronotropic actions of ET-1. Further examinations, including the dual blockade of ETA and ETs receptors and measurement of protein expression related to the calcium handling, are needed to assess the hemodynamic improvements with A-192621 treatment. Histologically, the degeneration of cardiomyocytes observed in the J2N-k hamsters was worsened, with respect to size and degree, by treatment with A-192621 (Figs 3D, 4D, Table 2). Thus, ETs receptor blockade in J2N-k hamsters apparently increased LV function, but caused further deterioration of the histological damage in the LV myocardium.

Chronic treatment with the ETA antagonist, ABT-627, significantly decreased the ET-1 content in the LV myocardium, but treatment with A-192621 did not. The autocrine regulation of ET-1 production via the ETA receptors has been reported. In addition, it has been suggested that ETs receptors play an important role in the local cardiac clearance of ET-1 peptide, and that ET-1 bound to ETs receptors may be rapidly internalized and degraded. In effect, the decreased ETA-mediated feedback system for ET-1 production and the ETs-mediated clearance of ET-1 in the ABT-627 treatment group may have been counteractive.

In the present study, the J2N-k hamsters exhibited increased NADPH diaphorase activity in cardiomyocytes (Fig 3K) and we have previously shown that increased NADPH diaphorase activity in cardiomyocytes might be caused by iNOS. Indeed, in patients with IDC, both high activity and expression of iNOS have been observed in the myocardium. It has been reported that nitric oxide induced by cytokines produces negative inotropic and chronotropic effects on cardiac myocytes. Chronic treatment with ABT-627, but not with A-192621, suppressed the NADPH diaphorase activity in LV tissues from J2N-k hamsters. However, it is unclear from our results whether ET-1 directly or indirectly affects the NAPDH diaphorase activity or iNOS protein expression. One possible explanation is that the ET-1/ETA system in cardiomyocytes augments cytokine-induced iNOS expression by stimulating a protein kinase C pathway and/or a cAMP pathway, thus contributing to enhanced iNOS mRNA concentrations. Thus, the beneficial effects of ABT-627 might be related to decreased iNOS expression. Further investigations are required to elucidate the relationship between the ET-1/ETA system and iNOS protein expression in cardiomyopathy.

In conclusion, selective ETA antagonist, but not ETs antagonist, reduced the ET-1 content as well as the NADPH diaphorase activity and preserved the fine structure of the LV myocardium in J2N-k cardiomyopathic hamsters. Long-term treatment with ETs-selective antagonist might worsen the degeneration of myocardial cells through the ET-1/ETA system even if LV function is improved.

Acknowledgments

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