Plasma levels of the vasoconstrictor peptide endothelin (ET)-1, which is produced by cardiac myocytes and vascular endothelial cells, are increased in patients and animals with severe congestive heart failure (CHF) [1–4]. Chronic activation of the ET-1 receptor system occurs in CHF and therefore may contribute to the progression and severity of left ventricular (LV) dysfunction. Onishi et al. showed that the increased endogenous ET-1 depresses LV contractile performance of dogs with pacing-induced CHF [5]. Thus endogenous ET-1 would provide adverse hemodynamic effects in CHF.

Recently there is experimental evidence to suggest that interactions exist between the angiotensin (ANG) II and ET-1 receptor transduction pathways in LV hypertrophy [6–9]. It has been reported that the endogenous ET-1 is locally produced and secreted by cardiomyocytes through a mechanism of ANG II–induced cardiac hypertrophy via an autocrine/paracrine fashion in rats [10]. It has not been made clear in the setting of CHF, however, whether the production of ET-1 and the cardiac effects of endogenous ET-1 in CHF may be unaffected by ANG II acting through AT1 receptors. [Japanese Journal of Physiology, 51, 445–453, 2001]

Abstract: Interactions between angiotensin (ANG) II and endothelin (ET)-1 receptor transduction pathways have been unclear in congestive heart failure (CHF). Therefore the objects of this study are, in CHF, whether production of ET-1 is modulated by ANG II and/or whether hemodynamic effects of endogenous ET-1 are modulated by ANG II. Twelve dogs were randomly assigned to two groups: untreated (n=6) and treated with ANG II type 1 (AT1) receptor antagonist (TCV116, 1.5 mg/kg/d) (n=6). After rapid ventricular pacing (240 bpm) for 4 weeks, plasma and cardiac ET-1 levels were compared between the two groups. Acute hemodynamic effects of a nonspecific ETA&B receptor antagonist, TAK044 (3 mg/kg plus 3 mg/kg/h I.V.) were examined in both groups by a conductance catheter and a micromanometer. After 4 weeks of pacing, plasma and cardiac tissue ET-1 levels were elevated in both groups to a similar degree. In the group treated with TCV116, TAK044 produced an increase in stroke volume and a decrease in total systemic resistance; heart rate was unchanged. The time constant of left ventricular (LV) relaxation was significantly decreased. The slope of LV end-systolic pressure–volume relation (EES) was increased (p<0.05), indicating an increased LV contractility. Thus endogenous ET-1 produces an arterial vasoconstriction and impairs LV contractility and relaxation in CHF with AT1 receptor antagonism. These hemodynamic responses to TAK044 in CHF treated with TCV116 were similar in untreated CHF. These results suggest that the production of ET-1 and the cardiac effects of endogenous ET-1 in CHF may be unaffected by ANG II acting through AT1 receptors. [Japanese Journal of Physiology, 51, 445–453, 2001]

Key words: endothelin-1, angiotensin II, pressure–volume relation, congestive heart failure, dog.
time-dependent changes in LV and cardiomyocyte function, structure, hemodynamic compromise, and hormonal activation (including ET-1) that are similar to the clinical spectrum of CHF [11]. Therefore, a model of pacing-induced CHF was used to test the central hypothesis that in CHF, the production of ET-1 and the hemodynamic effects of endogenous ET-1 are modulated by ANG II. To avoid the potential confounding effects of ET-1–produced changes in loading conditions on the conventional measures of LV performance, we evaluated LV contractile performance by using the pressure–volume (P–V) plane in dogs.

**MATERIALS AND METHODS**

The study was approved by the Animals Research Committee of Mie University School of Medicine. Specific attention was given to the appropriateness of the animal model, the welfare of the animals, the adequacy of anesthesia, and the methods of instrumentation.

**Dose-selection studies.** To estimate the hemodynamic effects of endogenous ET-1, TAK044, a potent mixed ET-1 antagonist, was used. An appropriate dosing strategy for ET-1 receptor blockade produced by TAK044 was selected, using six dogs. After baseline hemodynamic measurements, ET-1 (300 ng/kg I.V.) was administered and data were collected. On the following days, the same dose of ET-1 was infused and data were acquired again in the same animals pretreated by TAK044 (3 mg/kg I.V., followed by 3 mg/kg/h I.V.).

**Pacemaker implantation.** The following project, which used a rapid-pacing model of CHF in dogs, has been described previously [12]. Briefly, permanent pacemakers were implanted in 12 healthy, adult, male, heartworm-negative mongrel dogs (weight, 18–26 kg). Before the implantation of pacemakers, 20 ml of blood was drawn from the venous catheter and the plasma was stored at 80°C until the assay. The dogs were anesthetized with ketamine (10 mg/kg I.M.) and sodium pentobarbital (25 mg/kg I.V.); supplemental doses were given as necessary to maintain anesthesia. A ventricular pacing lead was fixed into the apex of the right ventricle via the left external jugular vein and connected to a modified multiprogrammable pacemaker (8329, Medtronics, Inc.) buried in a subcutaneous pocket.

After full recovery from the instrumentation (10 to 14 d after surgery), rapid ventricular pacing (240 bpm) was initiated by use of an external magnetic control unit. At the 8th day after pacing, 12 dogs were randomly assigned to the following treatment protocols: (1) no treatment (n=6), and (2) treatment with ANG II type 1 (AT1) receptor antagonist (TCV116, 1.5 mg/kg/d, PO) (n=6). The drug treatment protocols were continued for the entire 4-week pacing protocols.

**Data collection and calculation.** On the day after the 4-week pacing period, the pacemaker was turned off and the animals were allowed to equilibrate for 30 to 40 min. After 20 ml of blood was drawn, the animals were anesthetized with alpha-chloralose (50 mg/kg I.V.) and urethane (600 mg/kg I.V.); supplemental doses were given as necessary to maintain anesthesia [12]. The dogs were allowed to breathe spontaneously and were kept in a right lateral position throughout the study. To obtain LV pressures and volumes, a 7 F micromanometer-tipped catheter (Millar Instruments) via the right femoral artery and 7 F conductance catheter (Webster Labs) via the right carotid artery were positioned at the LV apex under fluoroscopic guidance. The conductance catheter was connected to a digital stimulator microprocessor (Sigma 5, Leycom) to measure LV conductance and to convert it to LV volume. Real-time pressure–volume diagram generation and eight-channel analog/digital conversion at 200 Hz were measured by using a 16-bit microcomputer system (PC-9801VX, NEC Co., Tokyo, Japan). The LV volume obtained by the conductance method was calibrated by contrast ventriculography by using the area–length method. A femoral vein was cannulated with a balloon-tipped catheter to occlude the inferior venae cavae.

**Experimental protocol.**

**Acute effects of ET-1 blocker.** The acute hemodynamic effects of TAK044 were examined in CHF groups with and without TCV116. We collected three sets of steady-state and variably loaded P–V loops generated by gradual transient occlusion of the inferior venae cavae over 15 s. After hemodynamic variables were allowed to restabilize, 3 mg/kg and 3 mg/kg/h of TAK044 were infused intravenously. The steady-state and transient caval occlusion data were collected every 5 min after the infusion until a stable effect was present.

**Acute effects of nitroprusside.** On the next day, to assess the direct cardiac effect of ET-1 blocker, independent of its effect on systolic load, we compared the equal hypotension caused by nitroprusside and TAK044. Nitroprusside (0.5 to 2.0 ng/kg/min) was administered to obtain a similar decrease in LV end-systolic pressure (PES). Data were collected in a similar way.

After the hemodynamic studies, the heart was excised and the LV free wall was cut into 2 to 3 pieces. The tissue was placed in separate tubes, frozen in liq-
uid nitrogen, and stored at −80°C until the time of neurohumoral assay.

**Data processing and analysis.** The derivatives of LV pressure (+dP/dt) were calculated by using the five-point Lagrangian method [5, 12]. The LV P_{ES} and end-systolic volume (V_{ES}) data during the fall in pressure, produced by each caval occlusion, were fit by using the least-squares technique to P_{ES}=E_{ES}(V_{ES}−V_{0}), where V_{0} is an intercept with the volume axis. E_{ES} is sensitive to changes in a contractile state but relatively insensitive to changes in loading conditions [5]. Stroke work (SW) was calculated by point-by-point integration of the LV P−V loop for each beat. Stroke volume (SV) was calculated as LV end-diastolic volume (V_{ED}) minus V_{ES}.

The rate of LV relaxation was analyzed by determining the time constant of the isovolumetric fall of LV pressure (τ). LV pressure, from the time of peak negative rate of rise in LV pressure (−dP/dt) until a level 5 mmHg above the LV end-diastolic pressure (P_{ED}) of the next beat, was fit to an exponential equation: P=a·exp(−t/τ)+b, where P=LV pressure, t=time, and a and b are constants. The total systemic resistance (TSR) was calculated as P_{ES} divided by cardiac output. The effective arterial elastance (Ea) was calculated as P_{ES} divided by SV. The coupling of the LV and arterial system was quantified as E_{ES}/Ea.

**Neurohormonal assays.** The plasma and tissue ET-1 and ANG II levels were determined by radioimmunoassay (RIA) as previously described [13]. Plasma norepinephrine (NE) concentration was performed by high-performance liquid chromatography.

**Statistical analysis.** Statistical comparisons were made with Student’s t-test for paired observations and ANOVA with the Bonferroni method of multiple-paired comparisons as appropriate [5, 12]. Significance was accepted when p<0.05. Data for steady-state and plasma and tissue ET-1 and ANG II levels and plasma norepinephrine levels are expressed as mean±SD, and values for LV P−V relations are expressed as mean±SE.

**RESULTS**

**Dose-selection studies**

As shown in Table 1, the infusion of ET-1 produced significant increases in P_{ES}, P_{ED}, TSR, and E_{ES}. All the ET-1−induced effects were completely blocked by pretreatment with TAK 044. Thus TAK044 (3 mg/kg I.V. followed by 3 mg/kg/h I.V.) was used as an ET-1 receptor antagonism in this study.

**Effects of chronic AT1 receptor blockade in CHF**

At the baseline, all dogs in the study had hemodynamic data and angiographic findings that were within the normal limits for mongrel dogs in our laboratory (Table 2). The two groups had no significant differences between them in any variables.

A comparison of the hemodynamic changes in the dogs after CHF between those treated and those untreated with TCV116 is summarized in Table 3. No significant changes in heart rate, V_{ED}, P_{ED}, and E_{ES} were noted between two groups. SV and E_{ES}/Ea was significantly higher, and TSR and Ea were lower in the group treated with TCV116 than in the untreated group. These findings indicate that TCV116 in CHF has no direct effect on LV contractility performance, but it does cause systemic arterial dilatation and improves arterial-ventricular coupling.

**Acute effects of ET-1 blocker in dogs with CHF**

Steady-state hemodynamic response produced by

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**Table 1. Effects of exogenous ET-1 on the steady-state hemodynamics and P−V relations with and without TAK044 before CHF.**

<table>
<thead>
<tr>
<th>Heart rate (beat/min)</th>
<th>LVP_{ES} (mmHg)</th>
<th>LVP_{ED} (mmHg)</th>
<th>Peak(+) dP/dt (mmHg/s)</th>
<th>TSR (mmHg/ml/min)</th>
<th>SV (ml)</th>
<th>E_{ES} (mmHg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>107.6±1.6</td>
<td>109.4±7.4</td>
<td>5.8±1.1</td>
<td>2.168±125</td>
<td>0.034±0.001</td>
<td>29.5±2.0</td>
</tr>
<tr>
<td>ET-1</td>
<td>104.5±1.9</td>
<td>119.2±7.3*</td>
<td>10.2±1.3*</td>
<td>2.256±131</td>
<td>0.039±0.001*</td>
<td>29.1±2.1</td>
</tr>
<tr>
<td>TAK044</td>
<td>108.3±1.7</td>
<td>107.8±6.1</td>
<td>4.9±0.8</td>
<td>2.139±127</td>
<td>0.032±0.001</td>
<td>31.4±2.3</td>
</tr>
<tr>
<td>ET-1</td>
<td>107.6±1.1</td>
<td>108.9±7.1</td>
<td>5.1±0.8</td>
<td>2.150±125</td>
<td>0.034±0.001</td>
<td>29.9±1.8</td>
</tr>
</tbody>
</table>

LV, left ventricle; LVP, LV pressure; dP/dt, derivatives of left ventricular pressure; TSR, total systemic resistance; SV, stroke volume; E_{ES}, the slope of linear P_{ES}−V_{ES} relation. Values are mean±SEM (n=5). *p<0.05, ET-1 versus corresponding control value.
TAK044 in untreated CHF is summarized in Table 3 and displayed in Fig. 1. TAK044 produced a decrease in $P_{ES}$, $P_{ED}$, $E_a$, TSR, and $t(\rho<0.05)$ and an increase in SV ($\rho<0.05$) without a change in heart rate, indicating marked systemic arterial dilatation. A typical example of the effect of TAK044 on variably loaded $P$–$V$ relations in CHF is shown in Fig. 1. TAK044 produced a markedly leftward shift of the end-systolic $P$–$V$ relation with an increased slope of $23\%$ (Table 3, $\rho<0.05$). This result shows that blocking endogenous ET-1 by TAK044 produces marked augmentation of LV contractility in CHF.

TAK044 in untreated CHF is summarized in Table 3 and displayed in Fig. 1. TAK044 produced a decrease in $P_{ES}$, $P_{ED}$, $E_a$, TSR, and $t(\rho<0.05)$ and an increase in SV ($\rho<0.05$) without a change in heart rate, indicating marked systemic arterial dilatation. A typical example of the effect of TAK044 on variably loaded $P$–$V$ relations in CHF is shown in Fig. 1. TAK044 produced a markedly leftward shift of the end-systolic $P$–$V$ relation with an increased slope of $23\%$ (Table 3, $\rho<0.05$). This result shows that blocking endogenous ET-1 by TAK044 produces marked augmentation of LV contractility in CHF.

**Acute effects of ET-1 blocker in dogs with CHF treated with AT1 receptor antagonist**

The steady-state hemodynamic response produced by TAK044 in CHF treated with TCV116 is summarized in Table 3 and displayed in Fig. 2. TAK044 produced a decrease in $P_{ES}$, $E_a$ and TSR and an increase in SV ($\rho<0.05$) with no changes in heart rate. TAK044 caused a significant decrease in $t$ and $P_{ED}$ and an increase in peak($-1$) $dP/dt$. There was no significant difference in the hemodynamic effects of TAK044 between two CHF groups treated and untreated with TCV116. A typical example of the effect of TAK044 on variably loaded $P$–$V$ relations in CHF treated with TCV116 is shown in Fig. 2. TAK044 produced a markedly leftward shift of the $P$–$V$ relation with an increased $E_{ES}$ of $24\%$. This result shows that blocking endogenous ET-1 increases LV contractility in CHF treated with TCV116 to an extent similar to untreated CHF, indicating that cardiac effects of endogenous ET-1 were unaffected by chronic AT1 receptor antagonism in CHF.

**Effects of nitroprusside after CHF with or without chronic AT1 receptor antagonism**

As shown in Table 4, nitroprusside decreased $P_{ES}$

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**Table 2. Hemodynamic characteristics of dogs before pacing.**

<table>
<thead>
<tr>
<th></th>
<th>Untreated group</th>
<th>TCV116 group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>Heart rate (beat/min)</td>
<td>106.2±1.1</td>
<td>110.7±2.3</td>
</tr>
<tr>
<td>$LVP_{ES}$ (mmHg)</td>
<td>114.4±6.9</td>
<td>108.8±6.4</td>
</tr>
<tr>
<td>$LVP_{ED}$ (mmHg)</td>
<td>7.2±1.0</td>
<td>6.0±1.0</td>
</tr>
<tr>
<td>$P_{ES} (\text{mmHg})$</td>
<td>2,116±121</td>
<td>2,199±186</td>
</tr>
<tr>
<td>$TSR$ (mmHg/ml/min)</td>
<td>0.034±0.001</td>
<td>0.031±0.001</td>
</tr>
<tr>
<td>$LV_{ED}$ (ml)</td>
<td>66.8±2.3</td>
<td>65.9±1.7</td>
</tr>
<tr>
<td>$E_{ES}$ (mmHg/ml)</td>
<td>3.6±0.4</td>
<td>3.5±0.3</td>
</tr>
</tbody>
</table>

LV, left ventricle; LVP, LV pressure; LVV, LV volume; $dP/dt$, derivatives of left ventricular pressure; TSR, total systemic resistance; $E_{ES}$, the slope of linear $P_{ES}$–$V_{ES}$ relation. Values are mean±SD ($n=6$).
to an extent similar to TAK044. Compared with TAK044, however, nitroprusside produced no significant increases in EES in either group, with or without TCV116.

Neurohumoral system activity

Plasma hormonal characteristics before and after pacing-induced CHF dogs with and without TCV116 were shown in Fig. 3. The pacing-induced CHF resulted in significant increases in plasma ET-1, ANG II, and NE levels \((p<0.05)\). During the evolution of CHF, TCV116 did not abolish the increases in plasma ET-1 and NE levels and resulted in a further increase in the plasma ANG II level \((p<0.05)\). The myocardial ET-1 and ANG II concentrations in normal dogs (from our pooled data from normal dogs) and pacing-induced CHF dogs with or without TCV116 were shown in Fig. 3. The myocardial ET-1 and ANG II levels in untreated CHF were also significantly higher than pooled normal data. The myocardial ANG II levels were significantly lower in the CHF group treated with TCV116 \((p<0.05)\) than in the untreated CHF.
In contrast, no significant difference in myocardial ET-1 concentrations was found between two CHF groups.

**DISCUSSION**

The goal of the present study was to determine whether the production of ET-1 is modulated by ANG II and whether the hemodynamic effects of endogenous ET-1 are modulated by ANG II in CHF. We showed in CHF with AT1 receptor antagonism that the plasma and tissue ET-1 levels were as high as those in untreated CHF. It is interesting that the acute administration of ET-1 blocker improved LV contraction and relaxation in CHF treated with AT1 receptor antagonist to an extent similar to untreated CHF. From the
unique results in this study, it is very unlikely that in CHF the production of ET-1 and the hemodynamic effect of endogenous ET-1 may be modulated by ANG II acting through AT1 receptors.

**Pacing-induced heart failure treated with an AT1 receptor antagonist.** Consistent with past reports, chronic rapid pacing causes LV dilation and pump dysfunction, accompanied with an activation of the renin-angiotensin system [5, 14]. Specifically, increased circulating levels of ANG II have been identified to occur with the development of LV dysfunction. An increase in stroke volume and a reduction in total systemic resistance in CHF treated with TCV116 suggest that ANG II contributes to the progression of CHF in this model. We showed that myocardial ANG II levels were lower in CHF treated with TCV116 than in untreated CHF. The reduction in myocardial ANG II levels in the TCV116 group quite likely reflect a reduced uptake of ANG II into myocardial cells as well as potentially reduced synthesis. However, the CHF group treated with TCV116 did not significantly improve the indexes of LV pump function. These are consistent with previous observations [14, 15]. McDonald et al. failed to show any benefits on ventricular remodeling after long-term treatment with the ANG II receptor antagonist DUP-532 in dogs [15]. They suggested that the prevention of bradykinin degradation is an important factor responsible for the antiremodeling effects of ACE inhibitors. Spinale et al. examined in pigs with pacing-induced CHF that monotherapy of valsartan had no favorable effects on LV function and geometry despite a reduction of total systemic resistance [14]. Thus monotherapy of the ANG II receptor antagonist may have little effect in preventing LV remodeling in this CHF model.

**Production of ET-1.** Increased plasma ET-1 has been found in experimental and clinical CHF [1–4]. We found that myocardial tissue ET-1 levels in CHF was increased, and so were plasma ET-1 levels. Shear stress and stretch stimulate endothelial cell production in ET-1 and may increase a conversion of big ET to ET-1 in CHF [16, 17]. The interaction between ANG II and ET-1 receptor transduction pathways shown in LV hypertrophy may contribute to the production of ET-1, though it has been unclear in CHF [6–9]. Both norepinephrine and ANG II (which were elevated in this CHF model) increase the expression of prepro-ET-1 mRNA in cultured endothelial cells [18, 19]. However, we found that chronic treatment with TCV116 did not quell the increases in plasma and tissue ET-1 levels in CHF, though it also did not increase myocardial ANG II levels. This suggests that ANG II may not directly modulate the production of endogenous ET-1 through an AT1 receptor pathway. Consistent with our results, Spinale et al. illustrated that a combined ACE inhibitor and ANG II receptor antagonist during chronic rapid pacing significantly reduced plasma ET-1, but it was not achieved by ANG II receptor antagonist alone [20]. Clavell et al. demonstrated that chronic ACE inhibitor abolished the increases in plasma and tissue ET-1 [21]. The possible mechanism of inhibiting local ET-1 activation may be due to the potentiality of bradykinin via nitric oxide and cGMP as well as ANG II inhibition. These results support the possibility that an AT1 receptor antagonist may not affect ET-1 production in the development of CHF. Recently, McEwan et al. demonstrated that a myocardial ET-1 system gene expression in vivo is modulated by the infusion of ANG II only when combined with an AT1 receptor blockade [22]. The production of ET-1 may be regulated by ANG II acting through ANG II type 2 receptors.

**Hemodynamic effects of endogenous ET-1.** To avoid the potentially confounding effects of endogenous ET-1 on loading conditions, we evaluated an LV contractile performance, using the $P–V$ plane. We found that an ET-1 blockade in CHF produced a significant improvement in LV contractile performance, as indicated by the increased $E_{ES}$. These effects are independent of the alterations of loading conditions because nitroprusside, which caused a similar decrease in end-systolic pressure, did not change LV $P–V$ relations. We cannot neglect the effect of an increased coronary blood flow by an ET-1 receptor blocker on an LV contractile function (Gregg's phenomenon) [23]. Onishi et al. showed in pacing-induced CHF dogs that an equally hypotensive dose of nitroprusside to an ET-1 receptor blocker produced a similar increase in a coronary blood flow without an increase in $E_{ES}$ [5]. Although we did not measure coronary blood flow, it appears that the beneficial effect of blocking ET-1 receptors is due to a removal of the direct inhibition by ET-1 of LV contraction and relaxation. In cardiomyocytes isolated from rats with isoproterenol-induced CHF, Suzuki et al. demonstrated that ET-1 directly decreases cardiomyocyte contractile performance associated with a significant decrease in the peak $[Ca^{2+}]$, transient [24]. In CHF, both protein kinase A– and protein kinase C–mediated signal transduction systems are disrupted [25, 26]. Moreover, there is a decreased stimulatory G protein, but an increased inhibitory G, protein [27]. Thus ET-1–induced activation of an altered protein kinase C–mediates pathway or G, protein may exacerbate the dysfunctional $Ca^{2+}$ homeostasis, which might account for the further impairment in myocardial contraction and re-
laxation that we observed in CHF.

In the present study, we observed that an ET-1 blocker caused similar cardiac responses between CHF treated and untreated with a chronic ANG II receptor antagonist. This suggested in CHF that ANG II acting through AT1 receptors might not directly modulate the cardiac effects of endogenous ET-1. In this study, chronic ANG II antagonism with TCV116 could not avoid an increase in plasma and cardiac ET-1 levels as well as plasma norepinephrine levels in CHF. Zhu et al. demonstrated that in the dog ventricular trabeculae, ET-1 did not affect the basal force of contraction, but it did produce a pronounced negative inotropic effect in the presence of isoproterenol [28]. Thus an increased plasma norepinephrine may contribute to the negative inotropic effect of endogenous ET-1 even in CHF treated with AT1 receptor antagonist.

**Study limitations.** Several methodological issues should be considered in interpreting our data. First is the experimental model of CHF. Although rapid pacing produces an animal model that closely mimics clinical congestive cardiomyopathy [29], we cannot be certain our results apply strictly to CHF and that they are not due to other causes.

Second, we used a mixed ETA and ETB receptor antagonist. Because ET-1 has two receptors—ETA and ETB, which are distributed in various tissues and may be involved in the cardiac responses [30, 31]—the extent to which endogenous ET-1 affects cardiac performance through each receptor type is not addressed in our study. Future studies that compare cardiac effects between ETA and an ETA/ETB combined receptor blocker would be appropriate.

Because CHF typically involves long-term changes, it would have been of great interest to have had data on the effects of a long-term blockade of ET-1 receptors in CHF. However, we focused on the hemodynamic effects of endogenous ET-1 in CHF with chronic ANG II antagonism. To clarify the point, it is suitable to examine not long-term effects, but the short-term effects of an ET-1 blocker on hemodynamics in CHF with ANG II antagonism.

In our model, we instituted no method to avoid the autonomic influences on cardiac performance and hemodynamics. Nevertheless, heart rates were not significantly changed before or after acute injection of an ET-1 receptor blockade.

Another possible limitation is the difficulty in interpreting whether the benefits of TCV116 on LV function and chamber remodeling were due to vasodilatation alone or to a specific antagonism of ANG II. This issue could have been better resolved had the study contained another monotherapy in which a vasodilator was used that had been less likely to affect LV performance and remodeling.

**Conclusions.** The present study demonstrates that the plasma and tissue levels of ET-1 are increased in CHF with the chronic treatment of an AT1 receptor blockade as well as untreated CHF. Endogenous ET-1 has adverse effects on the LV function in CHF with a chronic treatment of AT1 receptor blockade as well as untreated CHF. From these unique results, it is very unlikely that the production and the cardiac effects of endogenous ET-1 in CHF may be directly modulated by ANG II antagonism acting through AT1 receptors.

We are grateful to Takeda Chemical Industries, Ltd., for their generous gift of TCV116 and TAK044. The authors acknowledge the technical assistance of Seiichiro Fukuyama, Kenji Matsumoto, Shinji Hane, Takashi Osawa, and Daisuke Kinugawa.

**REFERENCES**


