Acute Effect of Endothelin AB Antagonist on Sympathetic Outflow in Conscious Rats With Heart Failure

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Although ET-1 antagonists have been beneficial in the treatment of heart failure (HF), their involvement in the effect on the sympathetic nervous system in HF remains unknown. The present study investigated the role of endogenous endothelin (ET) in the sympathetic nervous system in HF by observing the effect of ET AB antagonist (TAK-044) on renal sympathetic nerve activity (RSNA) in conscious rats with HF (n=7). HF was induced by left coronary artery ligation and 6 weeks later, TAK-044 was intravenously administered in the conscious and freely moving rats. RSNA, mean arterial pressure (MAP) and heart rate were compared with rats with sham operations (sham; n=7). MAP was significantly decreased in both groups; however, RSNA was significantly decreased only in the HF group at 5 min after administration, and this change continued until 10 min. There was also an effect of TAK-044 on the arterial baroreflex function indicated by the slope of RSNA to the changes in MAP during phenylephrine and nitroprusside injection in both groups. Compared with the sham group, the HF group showed impaired arterial baroreflex control of RSNA during phenylephrine injection, and intravenous administration of TAK-044 normalized this abnormality, whereas the function in the sham group was not changed. These data show that ET AB antagonist suppressed renal sympathetic activity in rats with HF, and improved arterial baroreflex function. The beneficial effect of endothelin antagonist on heart failure may involve improvement of the increased sympathoexcitation and impaired arterial baroreflex function in HF. (Circ J 2002; 66: 841–845)

Key Words: Arterial baroreflex function; Myocardial infarction; Renal sympathetic nerve activity
Sympathetic Response Study

A renal nerve electrode was implanted under intraperitoneal sodium pentobarbital anesthesia. The left renal nerve was exposed through a flank incision and dissected from the surrounding tissue and renal artery. A pair of polytetrafluoroethylene-coated stainless steel wire electrodes (A-M Systems Inc) was placed around the dissected renal nerve. The electrodes and nerve were covered with a two-component silicone gel (Wacker Sil-Gel, Wacker Chemie, Munich, Germany). The ground lead was placed beneath the skin of the back. Simultaneously, catheters made of polyethylene (PE 10, fused with PE 50, Clay-Adams, NJ, USA) were inserted into the right femoral artery and vein for injection and measurement of mean arterial pressure (MAP) and heart rate (HR). The electrodes and catheters were tunneled beneath the skin to the back and fixed between the shoulder blades. At 24 h after implantation of electrodes, assessment of baroreflex sensitivity (BRS) was performed in conscious and freely moving rats. Renal sympathetic nerve activity (RSNA) was recorded after pre-amplification of the signal with a preamplifier (Nihon Kohden EL601G) with the band-pass filter set between 100 Hz and 1 kHz. The raw nerve activity was full-wave-rectified and integrated with a voltage integrator. Sympathetic nerve recordings had a signal-to-noise ratio of at least 3. RSNA is expressed as the percent change of resting activity for each experiment. After 30 min observation of resting values, we assessed the arterial BRS of RSNA, and 10 min after the first BRS assessment, the ETAB antagonist, TAK 044 (1 mg/kg), was administrated intravenously. The changes of MAP, HR and RSNA were observed for 10 min, and then the second BRS assessment was performed. The high dosage of TAK 044 was chosen to decrease the potent pressor effect of exogenously applied ET-1 (0.3 nmol/kg) by 50%. The postmortem noise was recorded after the animals had been killed by an overdose of pentobarbital.

### Table 1 Hemodynamic Measured by Left Ventricular Catheterization

<table>
<thead>
<tr>
<th></th>
<th>LVSP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>+dp/dt (mmHg/s)</th>
<th>–dp/dt (mmHg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF (n=7)</td>
<td>139±12</td>
<td>16±5*</td>
<td>5.952±360*</td>
<td>4.368±627</td>
</tr>
<tr>
<td>Sham (n=7)</td>
<td>124±12</td>
<td>4±1</td>
<td>8.031±204*</td>
<td>5.120±281</td>
</tr>
</tbody>
</table>

LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dp/dt, maximal rate of rise of left ventricular pressure; –dp/dt, minimal rate of fall of left ventricular pressure. Values are mean ± SEM. *p<0.05 compared with Sham.

### Table 2 Baseline Body Weight (BW), Mean Arterial Pressure (MAP), Heart Rate (HR) and Heart Weight to Body Weight Ratio

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>Heart weight body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF (n=7)</td>
<td>494±10</td>
<td>110±6</td>
<td>374±26</td>
<td>0.39±0.04</td>
</tr>
<tr>
<td>Sham (n=7)</td>
<td>500±10</td>
<td>102±3</td>
<td>398±12</td>
<td>0.29±0.02</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. *p<0.05, †p<0.01 compared with Sham.

Fig 1. Example of chart recordings of HR, AP and RSNA responses to TAK 044 (1 mg/kg) intravenous administration in a HF (A) and a sham-operated rat (B).

Fig 2. Time course of MAP (A), RSNA (B), and HR (C) after TAK 044 intravenous administration. Values are mean ± SEM. *p<0.05 compared with baseline value. †p<0.01 compared with baseline value.
BRs was analyzed by the slopes of the regression line of RSNA in relation to the change in MAP during 3 stepwise injections of phenylephrine (15–45 µg/kg) and nitroprusside (15–45 µg/kg). The range of increase of MAP was within 50 mmHg, and the range of decrease was within 30 mmHg. A straight line was fitted to the linear portion of the MAP vs RSNA (RSNA was expressed as the percent change from basal nerve activity) curve using regression analysis. The beginning of the linear portion was determined as the MAP value at which the response for 2 consecutive points had decreased by $>5\%$, and the end of the linear portion was determined as the MAP value at which the response for 2 consecutive points had decreased by $<5\%$. The slope obtained from the RSNA (%) vs MAP (mmHg) curve were used as the arterial BRs. Extra and postextrasystolic heart beats were excluded from the data analysis.

**Hemodynamic Study**

After the sympathetic response study, we inserted a 2.4F micromanometer (Millar Instruments, Houston, TX, USA) for measurement of LV pressure via the right carotid artery under intravenous pentobarbital (2.5–5 mg) anesthesia. The micromanometer was connected to a pressure transducer (Nihon Kohden AP641G), and HR was measured by cardiotachometer triggered by the ventricular pulse. Positive and negative LV dp/dt were measured by pressure-processor (Nihon Kohden EQ-601G).

**Data Analysis**

Data are expressed as mean±SEM. The differences between the HF and Sham rats were analyzed by a Scheffe’s procedure for post hoc test after one-way analysis of variance. The effects of TAK 044 in each group were analyzed by paired-t test. Statistical significance was assumed if $p<0.05$.

**Results**

**Results of the Hemodynamic Study**

Under pentobarbital anesthesia, LVEDP in the HF rats was significantly elevated compared with the Sham rats ($p<0.05$), whereas there were no significant differences between the 2 groups in LV systolic pressure or HR (Table 1). Positive LV dp/dt in the HF rats was significantly reduced compared with the Sham rats ($p<0.05$), but negative LV dp/dt was similar in both groups. In the conscious and freely moving condition, there were no significant differences between the 2 groups in body weight, MAP or HR (Table 2). The heart weight-to-body weight ratio tended to increase in HF rats, but the difference was not significant ($p=0.07$).

**Effects of TAK 044 on MAP, HR and RSNA in HF and Sham Rats**

In HF rats, after TAK 044 administration, all parameters started to decrease gradually and at 5 min after administration, MAP and RSNA significantly decreased compared with baseline (Figs 1, 2). These effects continued for more than 10 min after administration. In Sham rats, MAP was significantly decreased at 10 min, and HR at 5 min after administration, but the changes of RSNA were not significant compared with baseline (Fig 2).

**Discussion**

The present study demonstrates that ET AB antagonist suppresses RSNA and improves the arterial baroreflex control of RSNA in conscious rats with MI-induced HF, but has no effects on either RSNA or arterial baroreflex control of RSNA in sham operated rats. First, we showed the inhibitory action on sympathetic nerve activity of ET AB antagonist in HF rats. The HF state is characterized by an augmented sympathetic nervous system, and the available evidence suggests that several factors contribute to this, including depressed arterial and cardiopulmonary reflexes, increased endogenous angiotensin II, depressed central synthesis of nitric oxide, and other increased circulating or local humoral factors. It is known that ET has an effect on sympathetic activation in either the central or peripheral nervous system in normal animals. In a recent study, Ling et al demonstrated that MAP and RSNA in ET-1 deficient mice were significantly higher than in wild-type mice in basal conditions. Although the site of this effect of ET is unknown, tissue autoradiographic studies have shown the presence of specific binding sites for ET-1 in the rat and human brain stem involved in cardiovascular regulation, such as the nucleus tractus solitarius and ventrolateral medulla, basal ganglia, and cerebellum. Furthermore, immunoreactive ET-1 and ET-3, as well as their mRNAs, are present in the central nervous system of the rat. ET concentrations in normal cerebrospinal fluid are 7-fold greater than in the plasma in normal humans, and 3-fold in rats. Some studies on intracerebroventricular administration of ET-1 have shown pressor responses and sympathetic activation. With regard to ET antagonist, McConnell et al showed that chronic treatment with a specific ETA antagonist reduced the normal rise in plasma norepinephrine by 50%, seen during 4 weeks of rapid pacing in a canine.
model of HF\textsuperscript{13} and Liu et al showed that both the selective ET A antagonist, BQ 123, and ET AB antagonist, L-754, 142, caused a time-dependent reduction in RSNA in rabbits with HF\textsuperscript{14} These data suggest that TAK modulates the sympathetic nervous system by suppressing increased endogenous ET via an ET A receptor in the central nervous system rather than in the peripheral system. Further study is, however, needed to determine whether ET is increased in the central nervous system of HF rats.

Second, we also have shown an improvement of arterial baroreflex sensitivity by ET AB antagonist administration in HF rats. Although the origin of sympathoexcitation has not been clearly defined, earlier studies suggested that abnormal arterial baroreflex and cardiopulmonary reflex control of sympathetic outflow were responsible for the enhanced sympathoexcitation of HF. In a clinical study, the assessment of BRS is useful for post infarction risk stratification of worsening hemodynamic state and prognosis.\textsuperscript{29} In rats with MI, impaired arterial baroreflex function has been shown, but the mechanism has not been clarified.\textsuperscript{16,18} Although recent studies suggest that endogenous angiotensin II is a likely candidate,\textsuperscript{19,30,31} Some studies have also documented ET actions on BRS\textsuperscript{32,33} and those results are controversial because both inhibitory and facilitatory actions have been demonstrated. Factors such as dose, route of administration, state of consciousness of the animals, and whether agonist or antagonist is used may account for these discrepancies. Mosqueda-Garcia et al showed modulatory effects of ET on the baroreflex in the nucleus tracts solitarius in rats.\textsuperscript{34} They reported an increased gain of the baroreflex slope with the intra nucleus tracts solitary administration of 2 different ET A antagonists, BQ-123 and -610, but not with the ET B antagonist, BQ-78834. Their results suggest that the alteration of arterial BRS in HF rats was evoked by ET AB antagonist through an ET A receptor subtype in the brain stem. With regard to HF, Liu et al showed that chronic treatment with the ET AB antagonist, L-754, 142, improved the impaired arterial baroreflex function in rabbits with HF, and they concluded that enhancement of arterial baroreflex function may contribute to sympathoinhibition after ET-1 blockade in HF.\textsuperscript{14} Furthermore, they compared the effects of ET AB and ET A antagonist, and concluded that the observed effects were most likely mediated by the ET A receptor. The stimulation of ET B receptors has been associated with increased synthesis of nitric oxide, which is known to be sympathoinhibitory. Therefore, it is unlikely that the contribution of ET B receptor blockade is significant. However, Chapleau et al demonstrated that ET can reduce baroreceptor discharge at the carotid sinus level.\textsuperscript{15} Further study is needed to decide the actual site of the effect of ET on baroreflex function.

Many studies have found that the ET-1 concentration is increased in the plasma of HF patients, and the concentrations increase as the severity of HF increases.\textsuperscript{36–38} Namely, the circulating ET-1 concentration in HF is negatively correlated with the LV ejection fraction and positively correlated with New York Heart Association functional class, LVEDP and the extent of pulmonary hypertension.\textsuperscript{39} On the basis of these observations, it is suggested that ET may play an important role in the long-term pathophysiological changes induced by HF and that ET antagonists may be useful therapeutic agents. Teclink et al showed that oral administration of Bosentan, another dual ET AB antagonist, significantly reduced MAP in conscious HF rats.\textsuperscript{40} Acute effects of the BQ-123, an ET A antagonist, has been shown by Sakai et al who observed that intravenous infusion of this agent significantly decreased HR and LV +dP/dt in rats with HF, but had no effect on these hemodynamic parameters in sham-operated rats.\textsuperscript{5} We have also demonstrated previously a reduction of LVEDP and +dP/dt, with simultaneous decrease of RSNA, by intravenous TAK044 administration in anesthetized rats with HF.\textsuperscript{41} These data support the idea that ET is involved in the maintenance of both cardiac function and blood pressure in HF; however, several studies have been performed to determine whether long-term treatment with ET antagonist has beneficial hemodynamic and cardiovascular effects and improves the survival in HF. Mulder et al showed that long-term treatment with bosentan in a rat model of HF markedly increased survival, and the increase was associated with decreases in both preload and afterload and an increase in cardiac output as well as decreased cardiac hypertrophy, LV dilatation, and fibrosis.\textsuperscript{42} Spinale et al also reported the effects of chronic blockade of the ET A receptor by PD156707 in a pacing-induced rabbit model of HF.\textsuperscript{43} They investigated not only global LV function but also isolated myocyte function, and showed that isolated myocyte shortening velocity was normalized, and found reduced inotropic responsiveness of the myocyte to extracellular calcium ions and β-stimulation. These studies suggest that the mechanisms of the beneficial effects of ET antagonists are reduced afterload, increased myocardial blood flow, increased oxygen capacity after decreased pulmonary vascular resistance, decreased HR, inactivation of neurohumoral systems, and improved cardiac contractility. Improved contractility is probably induced by the maintenance of calcium ion homeostatic processes, but the effect on neurohumoral mediators, including the sympathetic nervous system, remains unclear. Our data suggest that sympathoinhibition may also be a mechanism of the beneficial effects of ET AB antagonist.

Study Limitations

First, we decided to study arterial BRS with a stepwise bolus administration of vasoactive agents. One potential limitation is that we could not determine the threshold portion of the baroreflex slope and the operating range because we had not defined the entire sigmoidal curve. We selected this method because it allowed us to evaluate quickly and accurately in a short time interval, with the rats in a conscious and freely moving state, which is clearly advantageous\textsuperscript{52} and to our knowledge, there is no evidence that this method yields results highly dissimilar from other methods of studying arterial BRS.

Second, we are assuming that RSNA reflects global changes in sympathetic nerve activity. It is possible that differences occur in sympathetic outflow to various vascular beds after ET AB blockade. On the other hand, it has been shown that changes in RSNA not only reflects change in the renal release of norepinephrine but also correlate with nerve activity to other beds.\textsuperscript{43,44} Changes in RSNA most likely reflect sympathetic outflow to many beds, but differences may exist in selective vascular beds.

Conclusions

The present study indicates that ET AB antagonist suppresses basal RSNA associated with decreases of MAP, and also improves the impaired arterial BRS in conscious
Effect of Endothelin Antagonist in Heart Failure

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References


