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Angiotensin AT_1 receptor-mediated attenuation of cardiac hypertrophy due to volume overload: involvement of endothelin

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Abstract

The role of angiotensin II via the angiotensin type 1 (AT_1) receptor in the development of volume overload cardiac hypertrophy was investigated in adult male Wistar rats with aortic insufficiency. We examined the effects of specific angiotensin AT_1 receptor blockade with losartan (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]-imidazole potassium) on left ventricular weight and left ventricular angiotensin II and endothelin-1 level to test the possibility that the cardiac action of angiotensin II may be mediated by endogenous endothelin-1. Moreover, to verify the possible involvement of endothelin-1, we measured left ventricular endothelin-1 levels during the hypertrophic process and evaluated the effect of the endothelin ET_A receptor specific antagonist, FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1*H*-indolyl)propionyl]amino-3-(2-pyridyl) propionic acid), on left ventricular weight. Two weeks after production of aortic insufficiency, left ventricular weight and left ventricular endothelin-1 concentration were markedly elevated in the rats with aortic insufficiency as compared with the sham-operated control rats, but left ventricular angiotensin II was not changed. Losartan (10 mg/kg/day p.o., 2 weeks) significantly reduced left ventricular weight and left ventricular endothelin-1 level in the rats with aortic insufficiency without affecting blood pressure and there was a significant positive correlation between left ventricular weight and left ventricular endothelin-1 content. Left ventricular endothelin-1 content correlatively increased to left ventricular weight during the development of left ventricular hypertrophy. The left ventricular hypertrophy was significantly attenuated by chronic treatment with FR139317 (10 mg/kg/day i.p.) for 2 weeks. These findings indicate that endogenous angiotensin II may contribute, via the angiotensin AT_1 receptor, to the development of volume overload cardiac hypertrophy and that endogenous endothelin-1 may mediate some of the hypertrophic effects of angiotensin II.

Keywords: Cardiac hypertrophy; Volume overload; Losartan; Angiotensin II; Endothelin-1; FR139317

1. Introduction

Cardiac hypertrophy is an adaptive reaction of cardiac myocytes to chronic pressure or volume overload, and is thought to be one of the crucial risk factors leading to cardiovascular disease (Levy, 1991). The mechanisms that produce cardiac hypertrophy are not fully understood. Previous studies have implicated a potential role for angiotensin II in pressure overload cardiac hypertrophy by using angiotensin converting enzyme inhibitors (Nagano et al., 1991; Baker et al., 1990; Linz et al., 1989) and angiotensin II receptor antagonists (Mizuno et al., 1992; Kojima et al., 1994). Cardiocytes are known to bind angiotensin II with high affinity (Sechi et al., 1992). Cardiac angiotensin II receptors have been divided into subtypes by their affinity for nonpeptide inhibitors into angiotensin type 1 (AT_1; sensitive to losartan) and type 2 (AT_2; sensitive to PD 123319). The angiotensin AT_1 receptor is principally responsible for almost all the angiotensin II-induced systemic actions studied thus far (Timmermans et al., 1993). We have already shown that cardiac angiotensin II may contribute to left ventricular hypertrophy due to volume overload in rats with aortic insufficiency by evaluating the effects of losartan (Ishiye et al., 1993). However, the precise mechanism by which the AT_1 receptor antagonist reduces cardiac hypertro-

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phy produced by volume overload is not fully elucidated.

An active area of research is focused on the role of growth factors, such as endothelin-1, in cardiac hypertrophy. Endothelin-1 is a powerful vasoconstrictor peptide (Yanagisawa et al., 1988) and is also a potent hypertrophic stimulus for cultured cardiomyocytes (Shubeita et al., 1990; Ito et al., 1991). As angiotensin II has been shown to induce the expression and secretion of endothelin-1 in cultured cardiomyocytes (Ito et al., 1993), it is reasonable to speculate that angiotensin II via the angiotensin AT_1 receptor is involved in the development of cardiac hypertrophy not only directly, but also indirectly by interacting with endothelin-1.

The objective of the present study was to verify the possibility that endogenous endothelin-1 is involved in cardiac hypertrophy via interaction with angiotensin II. We have determined the changes in cardiac and systemic angiotensin II and endothelin-1 levels and evaluated the effects of chronic treatment with the angiotensin AT_1 receptor specific antagonist, losartan (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]-imidazole potassium) (Timmermans et al., 1991) on these changes. We also assessed the possible role of endothelin-1 in left ventricular hypertrophy by studying the time course of changes in left ventricular endothelin-1 levels and their relationship with left ventricular hypertrophy and evaluating the effects of FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1*H*-indolyl)propionyl]-amino-3-(2-pyridyl) propionic acid), an endothelin receptor antagonist (Sogabe et al., 1993), on the development of cardiac hypertrophy in rats with aortic insufficiency.

2. Materials and methods

2.1. Animal preparation

Aortic insufficiency was produced in male Wistar rats (270–290 g) by using previously described techniques (Uematsu et al., 1989; Umemura et al., 1992) with some modifications. Each rat was anesthetized with sodium pentobarbital (50 mg/kg i.p.) prior to operation. A fluid-filled polyethylene catheter connected to a pressure transducer was inserted into the right common carotid artery and advanced to the aortic valve to produce aortic insufficiency by perforating the cusps, while simultaneously monitoring aortic pressure. Establishment of aortic insufficiency was confirmed by an increase in the aortic pulse pressure by more than 60% of the pre-operation value. Rats treated in the same way but without perforating the cusp served as sham-operated controls. After operation, the rats were housed in a temperature-, humidity- and light-controlled room with regular rat chow and water ad libitum. All experimental procedures were approved by the Ethical Committee of Hamamatsu University School of Medicine.

2.2. Drug treatment

Losartan was dissolved in distilled water and administered orally for 2 weeks (10 mg/kg/day, 1 ml/kg). Administration of losartan was started one day after the production of aortic insufficiency. Treatment with losartan (10 mg/kg/day) for 2 weeks neither lowered blood pressure nor reduced cardiac mass in normotensive Wistar rats (data not shown). FR139317 was dissolved in saline and administered (10 mg/kg/day) intraperitoneally for 2 weeks after operation via an Alzet 2ML2 minipump (Alza Corp., Palo Alto, USA) implanted at the time of operation. FR139317 (10 mg/kg/day) did not lower arterial blood pressure in normotensive Wistar rats (data not shown).

The left femoral artery was cannulated with a polyethylene tube under anesthesia with pentobarbital (50 mg/kg i.p.) for monitoring arterial blood pressure and pulse rate through a pressure transducer and a carrier amplifier (MPU EQ-601G and AP-620G, Nihon Kohden, Tokyo, Japan). After measurement of blood pressure, blood samples were collected from the abdominal aorta into tubes containing aprotinin (300 kallikrein inactivating units/ml) and Na_2EDTA (1 mg/ml), and plasma were stored for measurement of plasma angiotensin II content. Hearts were excised and divided into atria, right and left (including the septum) ventricles and weighed after washing in cold saline, then frozen in liquid nitrogen and stored at −70° C for subsequent determination of angiotensin II and endothelin-1 content.

2.3. Time-course study

On the next day and then 3, 7, 14, and 28 days after production of aortic insufficiency, 3–6 rats each from the control and aortic insufficient groups were killed with an overdose of pentobarbital. Hearts were treated and stored as described above for subsequent measurement of left ventricular endothelin-1 content. Blood samples were taken on the next day and 14 days after surgery for determination of plasma endothelin-1 content.

2.4. Analysis of cardiac and plasma angiotensin II and endothelin-1 content

Extraction of tissue angiotensin II and endothelin-1 was performed essentially according to the methods of Phillips and Stenstrom (1985) and Matsumoto et al.

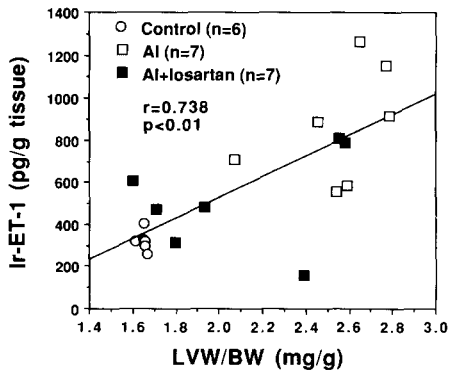

Fig. 1. Correlation between left ventricular weight (LVW/BW, mg/g) and left ventricular immunoreactive endothelin-1 concentration (Ir-ET-1, pg/g tissue) in rats with sham-operated (Control), aortic insufficiency (AI), and losartan-treated AI rats.

(1989). Frozen tissues were homogenized in 10 volumes of 1 N acetic acid containing 10 μg/ml of pepstatin and concentrated by passing through C-18 Sep-Pak cartridges (Waters, USA). Angiotensin II content was determined by radioimmunoassay using a radioimmunoassay kit (Nichols Institute, Netherlands) according to the manufacturer's recommendations. Endothelin-1 was measured with a radioimmunoassay using rabbit anti-endothelin-1 antiserum (IBL, Gumma, Japan) and ^{125}I-labeled endothelin-1 (Amersham Japan) as a tracer. Plasma samples were subjected to radioimmunoassay as described above.

2.5. Materials

Angiotensin II and endothelin-1 were purchased from Peptide Institute (Osaka, Japan). Aprotinin and pepstatin were purchased from Boehringer Mannheim. Losartan (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]-imidazole potassium) (Timmermans et al., 1991) was provided by Banyu Pharmaceutical Co., Tokyo, Japan. FR139317, (*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]-carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1*H*-indolyl)propionyl]amino-3-(2-pyridyl) propionic acid (Sogabe et al., 1993), was provided by Fujisawa Pharmaceutical Co., Osaka, Japan.

2.6. Statistical analysis

All values are expressed as the mean ± S.E.M. Statistical analysis was performed by analysis of variance (ANOVA) followed by Scheffe's multiple range test. Correlation between left ventricular weight and left ventricular immunoreactive endothelin-1 or angiotensin II concentrations was examined by linear regression analysis. An unpaired Student's *t*-test was used to assess the differences of left ventricular weight between control and aortic insufficient rats at each time point. A P value of < 0.05 was considered significant.

3. Results

3.1. Effects of losartan on the development of left ventricular hypertrophy

The effects of losartan on blood pressure, left ventricular weight, left ventricular angiotensin II and endothelin-1 level and plasma angiotensin II level are summarized in Table 1. Left ventricular weight, indexed as left ventricular weight/body weight (LVW/BW, mg/g), was significantly increased in the rats with aortic insufficiency as compared with the control rats. Treatment with losartan reduced LVW/BW significantly as compared with the untreated aortic insufficient rats. Systolic blood pressure did not change significantly within each group, and left ventricular and plasma angiotensin II concentrations did not change significantly as compared with the control group. Left ventricular endothelin-1 concentration was significantly elevated in aortic insufficient rats as compared with the control rats. Losartan treatment produced a marked reduction in left ventricular endothelin-1 concentration, but did not change left ventricular angiotensin II concentration, although it slightly increased plasma angiotensin II level.

Table 1
Left ventricular weight (LVW/BW, mg/g) and left ventricular immunoreactive angiotensin II and endothelin-1 concentration (pg/g tissue) in control, AI and losartan-treated AI rats

	Control (n = 6)	AI (n = 7)	AI + losartan (n = 7)
Systolic blood pressure (mm Hg)	135.1 ± 3.0	130.8 ± 4.8	140.4 ± 6.7
LVW/BW (mg/g)	1.65 ± 0.01	2.55 ± 0.09 [a]	2.08 ± 0.16 [b,c]
Left ventricular angiotensin II (pg/g tissue)	48.6 ± 4.5	56.1 ± 1.9	60.7 ± 4.2
Left ventricular endothelin-1 (pg/g tissue)	318.3 ± 21.0	866.8 ± 103.0 [a]	519.1 ± 84.1 [b,c]
Plasma angiotensin II (pg/ml)	53.7 ± 16.8	60.2 ± 17.1	89.7 ± 20.3

Control, sham-operated rats; AI, rats with aortic insufficiency. Values are presented as mean ± S.E.M. [a] $P < 0.01$ as compared with control rats. [b] $P < 0.05$ as compared with control rats. [c] $P < 0.05$ as compared with AI rats.

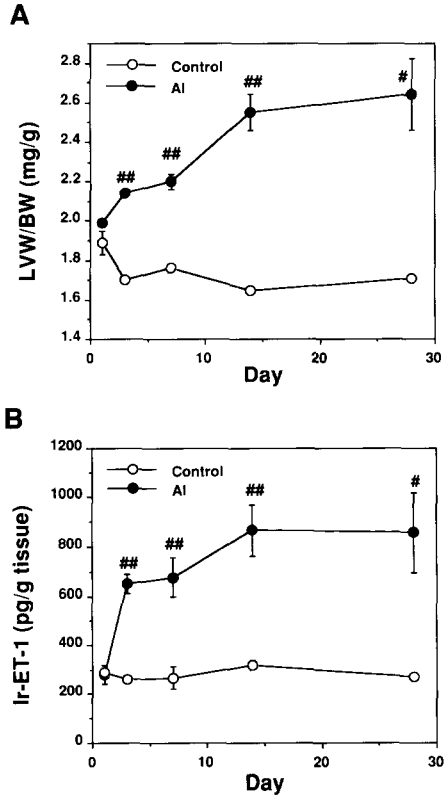

Fig. 2. (A) Time course of changes in left ventricular weight (LVW/BW, mg/g) in rats with aortic insufficiency (AI) and in sham-operated (Control) animals after surgical operation. Data points represent mean ± S.E.M. for 4–6 animals. (B) Time course of changes in left ventricular immunoreactive endothelin-1 concentration (Ir-ET-1, pg/g tissue) in rats with aortic insufficiency (AI) and in sham-operated (Control) animals after surgical operation. Data points represent mean ± S.E.M. for 4–6 animals. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus control animal at corresponding time point.

There was a significant positive correlation between left ventricular weight and left ventricular endothelin-1 concentration ($r = 0.738$, $P < 0.01$), as shown in Fig. 1. No significant correlation was observed between left ventricular weight and left ventricular angiotensin II concentration (data not shown).

3.2. Changes in left ventricular weight and left ventricular endothelin-1 concentration

Animals were killed at 1, 3, 7, 14, and 28 days after surgery. There was no significant difference between

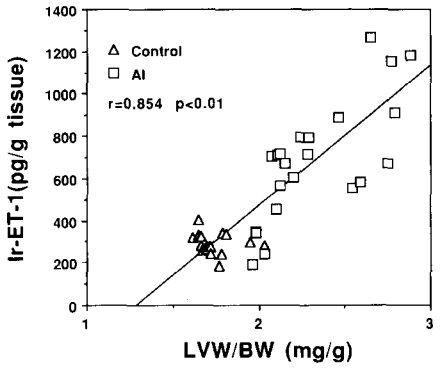

Fig. 3. Correlation between left ventricular weight (LVW/BW, mg/g) and left ventricular immunoreactive endothelin-1 concentration (Ir-ET-1, pg/g tissue) in rats with aortic insufficiency (AI) and sham-operated (Control) animals.

the aortic insufficient and control groups in body weight up to 4 weeks after surgery (data not shown). Fig. 2A shows the time course of the changes in left ventricular weight. Left ventricular weight began to increase 3 days after operation ($P < 0.01$). The mean left ventricular weight of the aortic insufficient rats was 2.51 ± 0.08 (mg/g) at 14 days after operation, which was significantly ($P < 0.01$) higher than that of the control rats and remained at about 2.5 up to 28 days after operation. Fig. 2B shows the changes in left ventricular endothelin-1 concentration during the development of volume overload cardiac hypertrophy. Concentration of left ventricular endothelin-1 began to increase from 3 days after operation and remained significantly ($P < 0.01$) higher than that of the control rats thereafter. To further evaluate the relationship between cardiac endothelin-1 level and the development of cardiac hypertrophy, regression analysis between left ventricular weight and left ventricular endothelin-1 was performed. Fig. 3 shows the correlation between left ventricular weight and left ventricular endothelin-1 concentrations. Left ventricular weight shows a strong correlation with left ventricular endothelin-1 concentration ($r = 0.854$, $P < 0.01$). Plasma concentration of endothelin-1 in the rats with aortic insufficiency was significantly higher than that of the control rats 1 day after surgery (6.2 ± 2.2 vs. 2.7 ± 1.1 pg/ml, $P < 0.05$), but there was no difference between the two groups at 14 days after operation (4.5 ± 0.8 vs. 4.6 ± 0.8 pg/ml).

Table 2
Effects of continuous infusion of FR139317 (10 mg/kg/day) on body weight, left ventricular weight (LVW/BW) and mean blood pressure (MBP) in AI rats

	Body weight (g)	LVW/BW (mg/g)	MBP (mm Hg)
AI ($n = 6$)	277 ± 5	2.48 ± 0.06	100.2 ± 2.6
AI + FR139317 ($n = 5$)	280 ± 5	2.14 ± 0.09 [a]	90.4 ± 0.7 [a]

AI, rats with aortic insufficiency. [a] $P < 0.05$ as compared with AI rats.

3.3. Effects of FR139317 on left ventricular hypertrophy

We further studied the possible involvement of endogenous endothelin-1 by evaluating the effect of FR139317, an endothelin ET_A receptor specific antagonist (Sogabe et al., 1993). The effects of FR139317 treatment are shown in Table 2. Left ventricular hypertrophy was significantly attenuated by the chronic treatment with FR139317. Although 10 mg/kg/day of FR139317 did not lower arterial blood pressure in normotensive Wistar rats, mean arterial blood pressure was slightly decreased in FR139317-treated aortic insufficient rats.

4. Discussion

In this study, using the rat as an experimental model, we have provided evidence that development of left ventricular hypertrophy due to volume overload is associated with an increase in left ventricular endothelin-1 level and that these changes can be prevented by specific angiotensin AT_1 receptor blockade with losartan. This appears to be a specific effect of losartan on the heart, as there was no difference in blood pressure between losartan-treated and untreated aortic insufficient rats. The increase in left ventricular weight during the development of cardiac hypertrophy showed a correlation with the increase in left ventricular endothelin-1 content. The increase in left ventricular weight was also suppressed following chronic treatment with FR139317, an endothelin ET_A receptor specific antagonist. These results suggest that cardiac endothelin-1 may mediate some of the hypertrophic effects of endogenous angiotensin II via the angiotensin AT_1 receptor in the development of volume overload cardiac hypertrophy.

Sadoshima et al. (1993) demonstrated, using an in vitro model of load-induced cardiac hypertrophy, that angiotensin II acts via angiotensin AT_1 receptors as an initial mediator of the hypertrophic responses, such as induction of immediate-early genes and fetal-type genes, and proposed that angiotensin II may trigger the subsequent autocrine/paracrine production of other secondary growth factors. Angiotensin II is known to stimulate endothelin-1 secretion from cultured endothelial cells (Emori et al., 1989) and from cardiomyocytes (Ito et al., 1993). Endothelin-1 is a 21 amino acids vasoactive peptide (Yanagisawa et al., 1988), and there are two types of endothelin receptor, ET_A and ET_B. The endothelin ET_A receptor is classified by having greater affinity for endothelin-1 over endothelin-3 and is believed to mediate the vasoactive and mitogenic responses, whereas the endothelin ET_B receptor is nonisopeptide selective (Sakurai et al., 1992). Hilal-Dandan et al. (1994) showed that adult rat ventricular myocytes express mainly ET_A receptors and the effects of endothelin on cardiomyocytes are long lasting. Endothelin-1 is also known to induce hypertrophy of cardiomyocytes (Ito et al., 1991; Shubeita et al., 1990). These data led us to speculate that the hypertrophic action of cardiac angiotensin II may be mediated, at least in part, by locally produced endothelin-1. Therefore, we evaluated the effects of blockade of angiotensin II and endothelin-1 receptors on left ventricular hypertrophy due to volume overload.

The present study confirms that left ventricular endothelin-1 content is increased in response to volume overload. In addition, we newly demonstrated that the increase in left ventricular endothelin-1 content can be inhibited by specific blockade of angiotensin AT_1 receptors. Attenuation of the increase in left ventricular endothelin-1 content in aortic insufficient rats with losartan treatment may indicate a possible link between angiotensin II action, via angiotensin AT_1 receptors, and endothelin-1 production. These findings indicate that the antihypertrophic effects of losartan on left ventricular hypertrophy are likely to be mediated by reduction of endothelin-1 production, and support our assumption that endothelin-1 is also involved in cardiac hypertrophy due to hemodynamic overload via endothelin ET_A receptors in response to angiotensin II. However, it is not clear from our study where the increase in endothelin-1 occurs. As endothelin-1 is produced in cardiomyocytes and endothelial cells in response to angiotensin II (Ito et al., 1993; Emori et al., 1989) and endothelin-1 is regarded as a local regulatory factor rather than a circulating hormone (Yanagisawa, 1994), and furthermore, production of endothelin-1 is enhanced by hypoxia in both types of cells (Kourembans et al., 1991; Kagamu et al., 1994), endothelin-1 would presumably accumulate in the interstitial space, when the heart became ischemic as a consequence of left ventricular hypertrophy. Released endothelin-1 may contribute to left ventricular hypertrophy by stimulating the endothelin ET_A receptor existing on the surface of cardiomyocytes (Hilal-Dandan et al., 1994) via stimulation of protein synthesis (Sugden et al., 1993). Alternatively, an increase in left ventricular endothelin-1 concentration may be a compensatory adaptation of the cardiomyocytes, because endothelin-1 is also known to produce a positive inotropic effect on ventricular myocytes (Takanashi and Endoh, 1991). Recently, Bakris et al. (1994) demonstrated that angiotensin converting enzyme inhibitors and losartan attenuated the proliferation of mesangial cells through a reduction of endothelin-1 production. These observations support the concept that some growth factors, such as angiotensin II and endothelin-1, interact to modulate cellular functions.

To obtain an insight into the possible involvement of endogenous endothelin-1 in cardiac hypertrophy, we

deduced the relationship between left ventricular weight and cardiac endothelin-1 level by measuring their changes during the development of left ventricular hypertrophy. In aortic insufficient rats, left ventricular mass began to increase from 3 days after production of aortic insufficiency as compared with the control rats, and was associated with an increase in left ventricular endothelin-1 concentration. However, plasma concentration of endothelin-1 in the aortic insufficient rats was transiently elevated soon after surgical operation and then returned to the same level as the control rats within 2 weeks. The results indicate that circulating and local endothelin systems are regulated by different mechanisms. These observations are in line with endothelin-1 as a local regulatory factor. Furthermore, the possible involvement of cardiac endothelin-1 via endothelin ET_A receptors in the present model was verified by the antihypertrophic effect of FR139317 treatment. The specificity of FR139317 to the endothelin ET_A receptor has been examined; FR139317 showed a high affinity for cloned endothelin ET_A receptors ($K_i = 1$ nM) and a lower affinity for cloned endothelin ET_B receptors ($K_i = 7.3$ μM) (Aramori et al., 1993) and inhibited the endothelin-1-induced contractions in isolated rabbit aorta with a pA_2 value of 7.2 (Sogabe et al., 1993). The continuous infusion of FR139317 used in this study maintained sufficient plasma concentration of FR139317 to block the pressor response to exogenously administered endothelin-1 (3.3 μg/kg i.v.) in normal Wistar rats (unpublished results). Further, although FR139317 did not lower blood pressure in normotensive rats, arterial mean blood pressure decreased in FR139317-treated aortic insufficient rats. This finding suggests that endogenous endothelin-1 may be involved in maintaining basal blood pressure in this model. We speculate that the anti-hypertrophic effect of FR139317 observed in this study was caused by both blockade of cardiac endothelin ET_A receptors and reduction in hemodynamic afterload.

Ito et al. (1994) demonstrated that BQ123, cyclo(D-Asp-L-Pro-D-Val-L-Leu-D-Trp), another endothelin ET_A receptor antagonist, blocked cardiac hypertrophy provoked by pressure overload at 1 week after aortic banding, but this effect was no longer observed at 2 weeks after aortic banding. They also showed that left ventricular prepro-endothelin-1 mRNA level and plasma endothelin-1 level increased transiently after aortic banding, and then decreased to the basal level within 4 days. From these observations they speculated that cardiac endothelin-1 may act during the early phase of cardiac hypertrophy due to pressure overload. However, we demonstrated in the present study that FR139317 attenuated left ventricular hypertrophy even at 2 weeks after production of aortic insufficiency. We assume, based on our present data, that cardiac endothelin-1 plays an important role during both the initial and maintaining phase of cardiac hypertrophy due to volume overload. This discrepancy between the effects of the two endothelin ET_A receptor antagonists on cardiac hypertrophy can probably be explained by the different properties of the models used. Moalic et al. (1981) reported that protein synthesis rates are different between these two models during the development of cardiac hypertrophy. They showed that protein synthesis rate transiently increased soon after aortic banding, but gradually increased in rats with aortic insufficiency. These changes in protein synthesis rate are coincident with the changes in cardiac endothelin-1 level during the development of cardiac hypertrophy due to volume overload. Therefore, it is likely that endothelin-1 is involved in the mechanisms of cardiac hypertrophy through stimulation of protein synthesis.

In conclusion, we demonstrated in this study for the first time that left ventricular endothelin-1 concentration was significantly increased in a rat model of volume overload cardiac hypertrophy, and showed that an angiotensin AT_1 receptor specific antagonist attenuated left ventricular hypertrophy, which was associated with a significant reduction of cardiac endothelin-1 level. The involvement of endogenous endothelin-1 in our model was verified by the effect of the endothelin ET_A receptor specific antagonist, FR139317. These findings suggest the possibility that left ventricular angiotensin II contributes to the development of volume overload cardiac hypertrophy via the angiotensin AT_1 receptor not only directly, but also indirectly by interacting with left ventricular endothelin-1, and that endothelin-1 induces cardiac hypertrophy in an autocrine/paracrine fashion via the endothelin ET_A receptor.

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