Original Article

Angiotensin II Type 2 Receptor Blockade Partially Negates Antihypertrophic Effects of Type 1 Receptor Blockade on Pressure-Overload Rat Cardiac Hypertrophy

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We investigated the effects of angiotensin II type 2 (AT2) receptor blockade on the antihypertrophic effects of type 1 receptor (AT1) blockade in pressure-overload cardiac hypertrophy in adult rats. Cardiac hypertrophy was induced by banding the abdominal aorta above the renal arteries. The rats were treated with either an AT1 receptor antagonist TCV-116 (TCV, 10 mg/kg/day), an AT2 receptor antagonist PD123319 (PD, 20 mg/kg/day), or both for 4 weeks after the aortic banding. We measured systolic and diastolic blood pressure (BP), body weight (BW), left ventricular weight (LVW), and serum and cardiac angiotensin converting enzyme (ACE) activities. Aortic banding increased BP and LVW/BW, and TCV reversed both these increases. PD affected neither BP nor LVW/BW. TCV + PD reversed the increase in BP but not LVW/BW. Thus, PD was considered to counteract the antihypertrophic effect of TCV without affecting BP. All three treatments reduced cardiac ACE activity without affecting serum ACE activity. Our data demonstrated that AT2 receptor blockade negates the antihypertrophic effects of AT1 receptor blockade in an adult rat model of pressure-overload cardiac hypertrophy. AT2 receptors may mediate the signaling pathways involved in growth inhibition, which could counteract mediation of the cellular growth signaling pathways by AT1 receptors. (*Hypertens Res* 2003; 26: 89–95)

Key Words: rat cardiac hypertrophy, pressure overload, angiotensin II type 1 receptor antagonist, angiotensin II type 2 receptor antagonist, angiotensin converting enzyme activity

Introduction

Each component of the renin-angiotensin system, including renin (1, 2), angiotensinogen (1), angiotensin converting enzyme (ACE) (3–5), angiotensin II (Ang II) (6) and Ang II receptors (3, 7), has been shown to be present in the rat heart, and each of these components has been shown to be upregulated in cardiac hypertrophy (4, 8–11). It has also been shown that cardiac hypertrophy can be effectively blocked by ACE inhibitors (4, 8) and Ang II type 1 (AT1) receptor

antagonists (12–14). These findings suggest that the cardiac renin-angiotensin system plays a crucial role in the development of cardiac hypertrophy.

AT1 and Ang II type 2 (AT2) receptors are the two main Ang II receptor subtypes, both of which may be upregulated in response to hypertrophic change in cultured rat cardiomyocytes (15) as well as in isolated rat hearts (11, 16, 17). Previous studies have reported that the numbers of each subtype of receptors are equally increased in hypertrophied cardiomyocytes, such that the ratios of these subtypes are maintained as in the normal myocardium (7, 11). On the other

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hand, the upregulation of AT₂ receptor may be associated with the downregulation of AT₁ receptor in pressure overload-induced cardiac hypertrophy (17). Nevertheless, the majority of the effects of Ang II on cardiovascular, hormonal and nervous systems, which induce hypertension and tachycardia, vasoconstriction, diastolic dysfunction of hypertrophied left ventricle, water drinking, adrenal aldosterone and catecholamine secretion, and release of catecholamine from sympathetic ganglia, *etc.*, are believed to be mediated by the AT₁ receptor (17–19).

Ang II has been suggested to inhibit proliferation of R3T3 fibroblast cells through the AT₂ receptor (20). The inhibition of neointimal formation in the balloon-injured carotid artery has also been reported to be mediated by the AT₂ receptor (21). In isolated perfused hypertrophied rat hearts, AT₂ receptor blockade has been shown to increase new cardiac protein synthesis, as determined by assessing the increased titrated phenylalanine incorporation in response to Ang II (22). Moreover, the cellular mechanisms of AT₂ receptor-mediated cell growth inhibition have been reviewed by Xoriuchi *et al.* (23). And Hiroi *et al.* recently reported that AT₂ receptor may be involved in the Ang II-induced negative regulation of the mitogen-activated protein kinases in cultured cardiac myocytes (24). However, the effects of chronic AT₂ receptor blockade on the development of cardiac hypertrophy in adult rats are not completely understood. In the present study, we investigated the effects of chronic AT₂ blockade on the development of pressure-overload cardiac hypertrophy and on the antihypertrophic effects of chronic AT₁ blockade in rats.

Methods

This study was approved by the Animal Ethics Committee of the Nagoya University School of Medicine.

Animals and Surgical Procedures

Experiments were performed using male Wistar rats (190–230 g). Cardiac hypertrophy was induced by banding the abdominal aorta above the renal arteries under anesthesia with pentobarbital sodium (50 mg/kg, *i.p.*). A stainless-steel wire (external diameter, 1.0 mm) was placed along the aorta and both the aorta and the wire were tied with a 3-0 silk thread. The wire was removed, leaving an aortic lumen determined by the diameter of the wire. Sham-operated animals were subjected to the same procedure without the aortic banding. Osmotic Alzet minipumps (2ML4; Alza, Palo Alto, USA) were implanted subcutaneously in the dorsum for the administration of an AT₂ receptor antagonist.

Experimental Protocol

The animals were divided into 5 groups as follows: i) a sham-operated group ($n = 7$) in which animals underwent sham-operation and vehicle treatments for TCV-116 (isotonic saline

and PD123319 [polyethyleneglycol (PEG)/isotonic saline, 1:1]; ii) a banding group ($n = 7$) that underwent aortic banding and vehicle treatments for TCV-116 and PD123319; iii) a banding with TCV group ($n = 7$) that underwent aortic banding, TCV-116 treatment (10 mg/kg/day) and vehicle treatment for PD123319; iv) a banding with PD group ($n = 7$) that underwent aortic banding, PD123319 treatment (20 mg/kg/day) and vehicle treatment for TCV-116; and v) a banding with TCV + PD group ($n = 6$) that underwent aortic banding and TCV-116 plus PD123319 treatment at the concentrations described above. TCV-116 was dissolved in isotonic saline and was administered by gavage daily for 4 weeks, starting on the day of the aortic banding. PD123319 was dissolved in PEG/saline (1:1) and infused subcutaneously by a miniosmotic pump for 4 weeks, also starting on the day of the aortic banding. The vehicle for TCV-116 was administered by gavage daily for 4 weeks, and that for PD123319 was infused subcutaneously by a miniosmotic pump for 4 weeks. After 4 weeks of each treatment, 24 h after the last drug administration by gavage, the animals were anaesthetized with pentobarbital sodium (30 mg/kg, *i.p.*), and were weighed after the removal of the miniosmotic pump. Blood pressure was measured *via* a catheter inserted into the right common carotid artery with a pressure transducer (DT-XX; Ohmeda, Englewood, USA) connected to an amplifier (Carrier Amplifier; Nihon Kohden, Tokyo, Japan) and recorded continuously for about 60 s on a Rectorider RJG-4124 (Nihon Kohden). Then, the maximum value of blood pressure was determined, and the heart rate was calculated from the pressure pulse interval. The blood was collected through the catheter into polyethylene tubes and centrifuged at $3,000 \times g$ for 10 min. The serum was stored at -70°C until the measurement of serum ACE activity. Immediately after blood sampling, the heart was excised and rinsed with ice-cold isotonic saline through the catheter inserted into the ascending aorta. The heart was blotted to dryness. The left ventricle was separated from both atria and the right ventricle. The ratio of left ventricular weight to body weight (LVW/BW, mg/g) was used as an index of cardiac hypertrophy. The left ventricles were stored at -70°C until the assay of cardiac ACE activity.

Serum and Cardiac ACE Activity

Serum and cardiac ACE activity were measured using a commercially available kit (ACE color; Fujirebio, Tokyo, Japan) containing *p*-hydroxybenzoyl-glycyl-L-histidyl-L-leucine as a synthetic substrate (25). The serum sample (100 μl) was incubated with the 10 mmol/l substrate (500 μl) at 37°C for 20 min, and another serum sample was incubated with the same amount of substrate in the presence of 3 mmol/l EDTA-2Na at 37°C for 20 min as a control. The reaction of ACE was stopped by adding 1.5 ml of a stopper/developer solution containing 3 mmol/l EDTA-2Na. The contents of the tube were mixed and reincubated at 37°C for 10 min to allow formation of index color quinoneimine dye

Table 1. Body Weight, Left Ventricular Weight and Heart Rate at 4 Weeks after Aortic Banding

	Body weight (g)	Heart rate (beats/min)
Sham (<i>n</i> = 7)	347 ± 8	362 ± 9
Banding (<i>n</i> = 7)	352 ± 6	412 ± 13
Banding with TCV (<i>n</i> = 7)	319 ± 8*	333 ± 18*
Banding with PD (<i>n</i> = 7)	343 ± 4	378 ± 20
Banding with TCV + PD (<i>n</i> = 6)	311 ± 6**.#	361 ± 14

TCV: TCV-116, PD: PD123319, sham: sham-operated group, banding: aortic banding group with vehicle treatments, banding with TCV: aortic banding group with TCV treatment, banding with PD: aortic banding group with PD treatment, banding with TCV + PD: aortic banding group with TCV + PD treatment. Mean ± SEM. * $p < 0.05$, ** $p < 0.01$ vs. Banding; # $p < 0.05$ vs. sham.

from the converted substrate. Serum ACE activity was calculated by the absorbance read at 505 nm. The frozen left ventricle was minced and homogenized with Polytron (KINEMATICA GmbH, Luzern, Switzerland) in 4 volumes of 50 mmol/l potassium phosphate-buffered saline, pH 7.5. The homogenate (100 μ l) was diluted with the substrate (500 μ l) and the measurements were performed exactly as described above, but with an initial incubation of 2 h.

Chemical Agents

TCV-116 was the gift of Takeda Chemical Ind. (Osaka, Japan). PD123319 was donated by Parke-Davis Pharmaceutical Research (Ann Arbor, USA). All other chemicals were purchased from Sigma Chemical (St. Louis, USA).

Statistical Analysis

All data are expressed as the means ± SEM. The differences between groups were assessed by one-way analysis of variance for parametric data and Kruskal-Wallis test for non-parametric data followed by Schéffe's test and Mann-Whitney *U* test, respectively. Values of $p < 0.05$ were considered to indicate statistical significance.

Results

Body Weight, Left Ventricular Weight and Heart Rate

The BW in the banding group was not different from that in the sham-operated group. The BW in the banding with TCV and the banding with TCV + PD groups were lower than that in the banding group, but that in the banding with PD group was not. Thus, the TCV and TCV + PD treatments reduced the banding-induced increase in BW. The heart rate in the banding group was not different from that in the sham-

operated group. The heart rate in the banding with TCV group was reduced compared to that in the banding group, but those in the banding with PD and the banding with TCV + PD groups were not (Table 1). Thus, the TCV treatment reduced the heart rate, and this reduction was reversed by the PD treatment.

Systolic and Diastolic Blood Pressures

The systolic and diastolic blood pressures (BP) in the banding group were higher than those in the sham-operated group. The BP values in the banding with TCV group and the banding with TCV + PD group were reduced compared with those in the banding group, but those in the banding with PD group were not (Fig. 1A). Thus, the TCV and the TCV + PD treatments prevented the aortic banding-induced increase in the BP, whereas the PD treatment did not.

Cardiac Hypertrophy

The LVW/BW was higher in the banding group than in the sham-operated group, indicating that banding successfully induced cardiac hypertrophy. LVW/BW was lower in the banding with TCV group than in the banding group, but there was no difference in the ratio among the banding with PD, banding with TCV + PD, and banding groups (Fig. 1B). In the banding with TCV + PD group, the LVW/BW was greater than that in the banding with TCV group. Thus, the TCV treatment prevented the aortic banding-induced increase in the LVW/BW, and this effect was inhibited by the PD treatment.

Serum and Cardiac ACE Activity

The banding had no effects on serum ACE activity, and there were also no differences in serum ACE activity among the experimental groups. The cardiac ACE activity in the banding group was greater than that in the sham-operated group. The cardiac ACE activities were significantly lower in the banding with TCV and the banding with PD than in the banding group, and that in the banding with TCV + PD seemed to be lower (Fig. 2). Thus, the TCV and the PD treatments prevented the aortic banding-induced increase in the cardiac ACE activity.

Discussion

Our present study revealed that chronic AT2 blockade antagonizes the antihypertrophic effects of chronic AT1 blockade, a result which suggests that AT1 and AT2 receptors play reciprocal roles in the development of pressure-overload cardiac hypertrophy in adult rats.

The doses of TCV-116 (10 mg/kg/day) and PD123319 (20 mg/kg/day) used in the present study have been reported to prevent the hypertension and cardiac hypertrophy induced by abdominal aortic banding (26) and to selectively block AT2

Fig. 1. Effects of TCV-116 (TCV), PD123319 (PD), and TCV-116 + PD123319 (TCV + PD) on systolic (open circles) and diastolic (closed circles) blood pressures (A) and the ratio of left ventricular weight to body weight (LVW/BW) (B). Sham, sham-operated group; Banding, aortic-banding group with vehicle treatments; Banding with TCV, aortic-banding group with TCV treatment; Banding with PD, aortic-banding group with PD treatment; Banding with TCV + PD: aortic-banding group with TCV + PD treatment. Data are expressed as the means $\pm$ SEM. ** $p < 0.01$ vs. Banding; ## $p < 0.01$ vs. Sham. $^{\dagger} p < 0.05$ vs. Banding with TCV.

receptors without affecting AT1 receptors (10, 21), respectively.

Our present study also demonstrated that the AT1 antagonist TCV-116 significantly prevented the increases in systolic and diastolic BP and LVW/BW. An interesting finding here is that the coadministration of AT1 and AT2 antagonists did not prevent cardiac hypertrophy but successfully protected the increase in BP after the aortic banding, indicating that the AT2 antagonist inhibited the antihypertrophic effect without affecting BP. Because AT1 receptor blockade is known to increase Ang II content (27), the AT1 antagonist may exert its antihypertrophic effect through the stimulation of AT2 receptors *via* the increase in Ang II. This hypothesis is further supported by the finding that the antihypertrophic effect of the AT1 antagonist was eliminated when both the AT1 and A2 receptors were blocked, or when the increased Ang II could not exert its antihypertrophic effect through the AT2 receptor.

It has been reported that AT1 receptor blockade is beneficial in the prevention of post-infarction increase in the right and/or left ventricular weight (28, 29). It has also been reported that AT2-receptor stimulation is beneficial for preventing the proliferation of cardiac fibroblasts in Bio 14.6 cardiomyopathic hamsters (30). Although AT1 blockade has been reported to alleviate post-infarction heart failure and reduce the infarct size in rats, and AT2 blockade has been reported to counteract these effects (31, 32), the present study is the first to examine these effects in hypertrophic hearts under prolonged periods of receptor blockade. Recently, AT2

receptor blockade has been shown to amplify new cardiac protein synthesis and protein kinase translocation in response to Ang II in isolated perfused hypertrophied rat hearts (22). However, the *in vivo* effects of chronic AT2 receptor blockade on the development of cardiac hypertrophy have not yet been investigated. Our present study was the first to demonstrate that chronic blockade of AT2 receptor negates the antihypertrophic effects of AT1 blockade, a finding which confirms and extends the previous findings in different rat models (28, 29, 31, 32).

The AT2 antagonist PD123319, when given alone, had no effect on the banding-induced cardiac hypertrophy. This result suggests that the hypertrophied rat heart caused by pressure overload did not respond to hypertrophic stimuli such as the AT2 receptor blockade. Schunkert *et al.* (33) reported that the acute responses of new protein synthesis to mechanical and neurohormonal stimuli were blunted in rat hearts with established left ventricular hypertrophy relative to non-hypertrophied control hearts. Accordingly, the hypertrophied hearts in our experiment can be assumed to have fully developed the hypertrophic response to the pressure overload produced by the aortic banding, and thus to have been unable to further respond to additional hypertrophic stimuli.

It is well known that the pressor response to Ang II is mediated by the AT1 receptor (19). However, the effects of the AT2 receptor on BP are still controversial. Ichiki *et al.* (34) and Hein *et al.* (35) have shown that AT2-deleted mice have an increased vasopressor response to Ang II, which may suggest that the AT2 receptor antagonizes the pressor effect

Fig. 2. Effects of TCV-116 (TCV), PD123319 (PD), and TCV-116 + PD123319 (TCV + PD) on serum and cardiac angiotensin converting enzyme (ACE) activities. Sham, sham-operated group; Banding, aortic-banding group with vehicle treatments; Banding with TCV, aortic-banding group with TCV treatment; Banding with PD, aortic-banding group with PD treatment; Banding with TCV + PD, aortic-banding group with TCV + PD treatment. Data are expressed as the means $\pm$ SEM. ** $p < 0.01$ vs. Banding; ## $p < 0.01$ vs. Sham.

of Ang II through the AT1 receptor, although in the former study the basal blood pressure was elevated and in the latter it was normal. Recently Goto *et al.* suggested that AT2 receptor expression is impaired in the spontaneously hypertensive rats (SHR) kidney (36). On the other hand, chronic blockade of the AT2 receptor by its specific antagonist PD123319 in Ang II-induced hypertensive rats had no effect on arterial BP in *in vivo* studies (37, 38), which is consistent with our results. Our findings in the present study suggest that the AT2 receptor may contribute at least in part to the regulation of arterial BP, although the precise mechanisms remain unclear.

In our present study, blockade of either AT1 or AT2 receptors significantly prevented the increase in cardiac ACE activity to a similar extent. However, only the AT1 blockade prevented the increases in BP and cardiac hypertrophy. These results suggest that the increased cardiac ACE activity may not primarily contribute to the development of cardiac hypertrophy in our experimental model. As a mechanism of the decreased cardiac ACE activity after the blockade of either AT1 or AT2 receptor, a negative feedback mechanism, previously demonstrated in the lung (39), may be functioning in rat hearts in response to the increased cardiac Ang II caused by AT1 or AT2 receptor blockade. An increase in Ang II concentrations following systemic administration of either an AT1- or an AT2-blocker has been shown in several studies (27, 40, 41). However, the different effects of the increased plasma Ang II on serum and cardiac ACE activities

remain to be elucidated.

Serum ACE activity was not affected by TCV-116 or PD123319 in the present study. Our results are in accordance with the previous studies which demonstrated that the AT1 receptor antagonists L-158809 and losartan did not change plasma ACE activity in Wistar rats (42) or SHR (43), respectively. One study (27), however, reported that losartan increased plasma ACE activity in Sprague-Dawley rats. The reason for this discrepancy may derive from the difference of the strains. However, the precise mechanism or/and the effect of AT1 antagonist on plasma or serum ACE activity remains to be elucidated.

A primary limitation of this study was that data on histology, molecular markers such as atrial natriuretic peptide (ANP), serum and cardiac Ang II concentrations, and cardiac function and geometry were very limited, although such information would have helped to clarify the underlying mechanisms involved in our results.

In conclusion, we demonstrated that an AT1 receptor antagonist, TCV-116, prevents pressure-overload cardiac hypertrophy in rats, and that this antihypertrophic effect may derive at least in part from the activation of AT2 receptors by the increased Ang II. Our findings suggest that AT1 receptor antagonists may constitute a useful therapeutic modality for patients with pressure-overload left ventricular hypertrophy.

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