Cardiac Angiotensin II Receptors Are Downregulated by Chronic Angiotensin II Infusion in Rats

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ABSTRACT—We studied the effect of chronic (7 days) angiotensin II infusion in a suppressor (200 ng/kg per minute) dose on angiotensin II receptors in the left ventricle in rats. Infusion of angiotensin II caused an elevation in systolic blood pressure after 3 days, usually to values of about 150 mmHg, and the increase continued during the drug administration. The number of angiotensin II type 1 and angiotensin II type 2 receptors was significantly decreased by 20–30% in the angiotensin II-infused left ventricular membranes without affecting the affinity. Thus, these data suggest that angiotensin II may regulate the number of its own receptors in rat left ventricles.

Keywords: Angiotensin II, Angiotensin receptor, Rat left ventricle

The angiotensin II receptor is a central component of the renin-angiotensin system. The action of angiotensin II mediated by its receptor causes a variety of effects such as elevation of blood pressure and cardiac hypertrophy and fibrosis (1, 2). Angiotensin II interacts with two pharmacologically distinct subtypes of cell-surface receptors, angiotensin II type 1 (AT1) and angiotensin II type 2 (AT2) receptors (3). AT1 receptors seem to mediate the major cardiovascular effects of angiotensin II, whereas the physiological roles of AT2 receptors are little known.

Angiotensin II has been shown to reduce AT1 receptor mRNA expression in cultured vascular smooth muscle cells (4), mesangial cells (5) and adrenal fasciculata cells (6). In contrast to these results, infusion of angiotensin II increased AT1 receptor mRNA expression in the adrenal gland but not that in aorta or kidney in vivo (7). Thus, tissue-dependent differences in the regulation of AT1 receptor may exist. In adult rat hearts, the levels of expression of AT1 and AT2 receptors are very low, but it was reported that the density of angiotensin II binding in rat hearts decreased after angiotensin II infusion (8). However, it is unclear whether the numbers of AT1 and AT2 receptors and the receptor affinity in the heart are changed in response to a chronic elevation in circulating angiotensin II. Therefore, in the present study, we investigated the effects of chronic angiotensin II infusion on the modulation of each subtype of angiotensin receptors in the rat left ventricle in vivo.

Experimental procedures in this study adhered to the Guiding Principles for the Care and Use of Laboratory Animals, approved by The Japanese Pharmacological Society. Twenty-week-old male Wistar-Kyoto rats, anesthetized with ether, were implanted subcutaneously with an Alzet osmotic minipump (model 2001; Alza Corp., Palo Alto, CA, USA) containing angiotensin II (Sigma, St. Louis, MO, USA) in saline or saline alone, and received a suppressor (200 ng/kg per minute) infusion of angiotensin II for 7 days. Systolic blood pressure and heart rate of each rat were measured by the tail-cuff method.

On the 7th day, the rats in each group were anesthetized by pentobarbital, the chest was opened, and then the heart was rapidly removed. Left ventricular membranes were prepared as described (9). In brief, the tissue was homogenized in 0.25 mM sucrose, 5 mM Tris, pH 7.5, 1 mM MgCl\textsubscript{2}, using a Polytron (Kinematica, Lucerne, Switzerland). The homogenate was sedimented at 500 × g for 15 min, and the supernatant was centrifuged at 50,000 × g for 30 min. The pellet was washed twice with incubation buffer (50 mM Tris, pH 7.5, 10 mM MgCl\textsubscript{2}), and then the final pellet was resuspended in incubation buffer containing 2 mg/ml bovine serum albumin (BSA) and 0.2 mg/ml bacitracin and stored at −80°C until used. The binding of angiotensin II to membrane suspensions was performed by incubating a membrane suspension (approximately 180–250 μg protein) with \textsuperscript{125}I-Sar\textsuperscript{1},Ile\textsuperscript{8}-angiotensin II (New England Nuclear, Boston, MA, USA) for 60 min at 25°C.
Nonspecific binding was assayed by competition with 1 μM unlabeled Sar₁, Ile₈-angiotensin II. AT1 and AT2 receptor subtypes were identified by competition with 10 μM PD123319, a selective AT2 receptor antagonist (Warner-Lambert, Ann Arbor, MI, USA), and CV11974, a selective AT1 receptor antagonist (Takeda Chemical Industries, Osaka), respectively. Reactions were terminated by addition of ice-cold incubation buffer. The reaction mixtures were rapidly filtered through a glass-fiber Whatman GF/B filter, using a Brandel 24R cell harvester (Brandel, Gaithersburg, MD, USA). The radioactivity trapped on the filters was quantitated with a gamma counter (ARC-360; Aloka, Tokyo). Each binding assay was carried out in triplicate. Protein concentrations of the samples were determined by the BCA protein assay (Pierce, Rockford, IL, USA). The maximum number of binding sites (Bₘₐₓ) and the affinity constant (Kᵢ) were determined by Scatchard analysis with the LIGAND program.

Data are expressed as the mean ± S.E.M. Comparison between the control and angiotensin II groups was performed by Student’s t-test. A P value of 0.05 or less was considered statistically significant.

Body weights and heart rates did not significantly differ between the two groups (data not shown). Infusion of angiotensin II caused an elevation in systolic blood pressure after day 3, usually to values of 150 mmHg, and the hypertension persisted over a 7-day period of infusion (Fig. 1).

A representative study is shown in Fig. 2 (A and B). ¹²⁵I-Sar¹, Ile₈-angiotensin II binding to membrane receptors was saturable in heart tissues of the control and angiotensin II group rats (Fig. 2A); Scatchard plot analysis revealed that ¹²⁵I-Sar¹, Ile₈-angiotensin II bound to a single class of receptors with high affinity (Fig. 2B). Although receptor affinity was similar in the two groups of rats (control, 1.55 ± 0.12; angiotensin II, 1.29 ± 0.11 nM), the number of receptors was decreased in the rats treated with angiotensin II. Binding of ¹²⁵I-Sar¹, Ile₈-angiotensin II in the presence of PD123319 and CV11974, which specifically inhibit AT1 and AT2 receptors, respectively, revealed the presence of both subclasses in rat hearts. In the angiotensin II group, the number (Bₘₐₓ) of both receptor subtypes was decreased on day 7 of angiotensin II infusion (Fig. 2C).

The regulation of angiotensin II receptors by angiotensin II depends on the tissue investigated. However, because of the low density of angiotensin II receptors in the rat heart, there was little known about the regulation of its receptors. We demonstrated herein that the chronic angiotensin II infusion decreases the number of angiotensin II receptors (both AT1 and AT2 receptors) without affecting the affinity constant in rat left ventricles (Fig. 2). The increase in blood pressure was noted on day 3 of angiotensin II administration.

It is important to consider the effects of hypertension on

Fig. 1. Time-related changes in systolic arterial pressure in the control and angiotensin II (200 ng/kg per minute) groups (n = 8, each group). *P < 0.05, **P < 0.01 versus control group.

Fig. 2. Radiolabeled receptor binding assay. A: Binding of ¹²⁵I-Sar¹, Ile₈-angiotensin II to heart membranes from a control rat (open squares) and from a rat administered angiotensin II for 7 days (closed circles). B: Scatchard plot analysis of data in A. C: Bar graphs showing the binding of ¹²⁵I-Sar¹, Ile₈-angiotensin II in the presence or absence of PD123319 or CV11974 in the control and angiotensin II groups (n = 4, each group). *P < 0.05 versus control group.
the expression of angiotensin II receptors. However, Ouali et al. (6) reported that the numbers of AT1 and AT2 receptors were downregulated by angiotensin II in cultured adrenal fasciculata cells. Our results are consistent with this in vitro study. Therefore, it is unlikely that the arterial hypertension induced by angiotensin II was responsible for the downregulation of angiotensin II receptors in the present study.

The mechanisms by which angiotensin II receptor is downregulated were not explored in this study. Nickenig et al. (10) reported that the number of AT1 receptors as well as the level of AT1 mRNA in atrial, ventricular and aortic tissues were downregulated in a rat model of high plasma renin and angiotensin II levels. In addition, several reports have shown that AT1 mRNA expression was downregulated by angiotensin II in cultured vascular smooth muscle cells (4), mesangial cells (5) and adrenal fasciculata cells (6). Thus, the decrease in angiotensin II receptor gene expression may be caused by angiotensin II in the present study.

In acute myocardial infarction, the AT1 receptor is upregulated (11), while it is decreased with development of heart failure. Indeed, Regitz-Zagrosek et al. reported that both AT1 and AT2 receptors were downregulated in failing human ventricles (12). In addition, cardiac ACE activity was increased in heart failure (13), suggesting that the enhanced production of angiotensin II may lead to a downregulation in the number of angiotensin II receptors in failing ventricles. Since angiotensin-converting enzyme inhibitors improve survival in patients with chronic congestive heart failure (14), a downregulation of the AT1 receptor can blunt the response of the failing heart to enhanced production of angiotensin II and is likely to be favorable in patients with heart failure.

In conclusion, this study demonstrated angiotensin II downregulates the number of its own receptors in rat left ventricles.

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