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# Cardiac Remodeling and Angiotensin II-Forming Enzyme Activity of the Left Ventricle in Hamsters with Chronic Pressure Overload Induced by Ascending Aortic Stenosis

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ABSTRACT. Cardiac remodeling and angiotensin II-forming enzyme activity of the left ventricle on chronic pressure overload were studied in male Syrian hamsters, whose chymase activity is similar to that of dogs. Pressure overload was achieved by banding at the ascending aorta (aortic stenosis). Echocardiography, histological analysis, and analysis of cardiac angiotensin-converting enzyme and chymase-like activities were performed. At 10 weeks after banding, concentric hypertrophy of the left ventricle was evident. At 20 weeks after banding, the ventricular weight-to-body ratio, cardiac fibrosis, and cardiac chymase-like activity were significantly increased, while cardiac angiotensin-converting enzyme activity was significantly decreased. This suggests that cardiac chymase, compared with cardiac angiotensin-converting enzyme, was activated against the chronic pressure overload and was responsible for the cardiac remodeling through the formation of angiotensin II. Considering the utility of the rodents, the interspecies similarity of the Ang II-forming pathway, and the effect of chymase in the hamsters, the present model is considered useful for studies evaluating the effect of Ang II and chymase in the canine heart with chronic pressure overload.

KEY WORDS: angiotensin-converting enzyme, chymase, hamster, remodeling, renin-angiotensin system.

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Cardiac remodeling can be described as the condition resulting from changes to the heart's cellular and molecular components [6]. This cardiac remodeling is controlled by mediators, such as the sympathetic nervous system and renin-angiotensin system (RAS). In the acute phase of cardiac dysfunction, the sympathetic nervous system and RAS are activated, inducing cardiac hypertrophy and increasing collagen synthesis in the cardiac interstitium (cardiac fibrosis), thereby enabling the heart to maintain function. This is considered an adaptive mechanism for cardiac dysfunction, and is regarded as either an adaptive phase or compensatory phase. Progressive remodeling, however, becomes maladaptive, leading to the progression of heart failure. Considering these mechanisms, all heart failures begin with cardiac remodeling, however, the processes occurring in cardiac remodeling are different from the underlying cardiac disease [6, 16].

Angiotensin II (Ang II) is produced by the cardiac RAS and directly activates cardiac myocytes [19, 35]. In rat models, Ang II is considered the cause of cardiac remodeling in pressure overload-induced heart failure [22, 34]. Therefore, suppression of the activation of Ang II is important and is effective in improving the prognosis for pressure overload-induced heart failure [10, 13, 27]. However, the rat is not an appropriate experimental animal for investigative studies into the relationship between cardiac RAS and cardiac remodeling in the dog because the Ang II-forming mechanism in the heart is different between rats and dogs [1, 11]. In the dog heart, cardiac Ang II is produced not only by cardiac angiontensin-converting enzyme (ACE) but also by cardiac chymase. However, Ang II is mainly produced by cardiac ACE in the rat heart [1, 20, 33]. On the other hand, the hamster is very useful because the Ang II-forming mechanism and the effect of chymase in the heart are similar to those of dogs [17].

In the present study, we focused on the mechanisms of development in heart failure under the conditions of chronic pressure overload using hamsters with ascending aortic stenosis. The cardiac ACE/chymase-like activities and cardiac hypertrophy/fibrosis were evaluated to demonstrate the relationship between cardiac RAS and cardiac remodeling in the heart with chronic pressure overload.

## MATERIALS AND METHODS

*Animal preparation*: Thirty-five 10 week old male Syrian hamsters (Japan SLC, Inc., Shizuoka, Japan) were used as a model of pressure overload. All animals were individually housed in a temperature-controlled room, maintained at 23 ± 2°C and 60 ± 10% humidity, with a 12/12-hr light/dark cycle. Commercial rat pellet (lab MR stock, Nihon Nosan Kogyo K.K., Kanagawa, Japan) and tap water were available *ad libitum*. The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

*Establishment of stenosis of the ascending aorta*: The chests of 19 hamsters were shaved, and then the animals

were positioned in dorsal recumbency under anesthesia with intraperitoneal pentobarbital (50 mg/kg). The bronchi were exposed via a midline incision. The animals were intubated with a 22-gauge intravenous catheter (Terumo, Tokyo, Japan), and artificial respiration was conducted. Anesthesia was maintained by inhalation of isoflurane (Isoflu, Dainippon Pharmaceutical, Osaka, Japan) and 100% oxygen. After shaving the ventral aspect of the chest, the midline on the sternum was opened with surgical scissors. A 22-gauge intravenous catheter was placed along the ascending aorta and banded together with the aorta using a 5–0 polypropylene suture (Prolene, Johnson and Johnson Medical, Tokyo, Japan). The catheter was then removed to establish aortic stenosis (group AS). After establishment of aortic stenosis, the thoracic cavity and skin were closed with a 4–0 nylon suture. An additional 16 animals underwent thoracotomy without placement of the ligation (group Sham). After respiration was stabilized, the catheter inserted into the bronchus was removed.

The body weight of the hamsters was measured biweekly. The hamsters underwent echocardiography preoperatively and postoperatively (10 and 20 weeks), and were sacrificed for cardiac morphometric examination and measurement of cardiac ACE and chymase-like activities at 20 weeks after banding.

*Serial echocardiographic assessment*: For the purpose of reducing the risk from anesthesia, echocardiographic measurements were randomly obtained for 6 hamsters from each group preoperatively and postoperatively (10 and 20 weeks). The chests of the hamsters were shaved and then the animals were positioned in left lateral recumbency under anesthesia with intraperitoneal ketamine hydrochloride (60 mg/kg) and xylazine (5 mg/kg). Echocardiography was performed using an ultrasonograph (Prosound SSD-5000, ALOKA Co., Ltd., Japan) equipped with a 10.0-MHz phase-array transducer (UST-979–3.5, ALOKA Co., Ltd., Tokyo, Japan). M-mode images were obtained from an optimized two-dimensional short-axis view of the left ventricle. Interventricular septal thickness, left ventricular posterior wall thickness (LVPWd and LVPWs, respectively), and left ventricular internal diameter in the diastolic and systolic phases (LVIDd and LVIDs, respectively) were measured in three consecutive cycles by a single observer and averaged. Left ventricular fractional shortening (FS) was calculated as (LVIDd-LVIDs)/LVIDd × 100. The ratio of left ventricle wall thickness to the internal radius in diastolic and systolic phases (LVPWd/(LVIDd/2) and LVPWs/(LVIDs/2), respectively) was calculated to indicate the presence of concentric hypertrophy.

*Heart isolation*: Under anesthesia with intraperitoneal pentobarbital (50 mg/kg), each hamster was anatomized using a median sternotomy, and potassium chloride (20 mmol/kg) was then injected into the left ventricle to achieve cardiac arrest in diastole. The heart was excised, and the blood was washed out with ice-cold saline. After the atrium was cut off, the ventricles were weighed, and the ventricular weight-to-body weight ratio calculated. The heart was cut cross-sectionally at the mid-papillary muscle level, and the apex side of the heart was frozen in liquid nitrogen and stored in a deep freezer at –80°C until measurement of ACE and chymase-like activities. The remaining hearts from each group were fixed in 10% formaldehyde and subjected to histological examination.

*Histological examination*: The ventricles were paraffin-embedded and cut into thin sections (3 $\mu$m) in the usual manner. They were then stained with hematoxylin-eosin and picrosirius red stain. The collagen density percentage was determined for 3 regions, the subendocardial, subepicardial, and middle portion of the left ventricle. Each region was assessed following staining with picrosirius red in 10 random fields with a magnification of × 200. The collagen density percentage was calculated using a computerized morphometry system (Mac Scope Ver 2.69.1, Mitani Co., Fukui, Japan), and the sum of all the areas stained positive for sirius red was divided by the sum of all myocardial areas for each hamster.

*Measurement of cardiac ACE and chymase-like activities*: Cardiac ACE and chymase-like activities were measured at 20 weeks after banding by high performance liquid chromatography as described previously [18]. One unit of ACE activity was defined as the amount of enzyme that produced 1 $\mu$mol of hippuric acid from Hippuryl-His-Leu per minute. After blocking Ang II-forming factors except chymase, cardiac chymase-like activity was defined by chymostatin-inhibitable Ang II formation and expressed as the amount of Ang II formed per g of tissue per minute.

*Drugs*: The following drugs were obtained from Sigma (St. Louis, MO, U.S.A.): angiotensin II acetate salt, aprotinin, *o*-phenanthroline, chymostatin, captopril, and hippuric acid hydrate. Angiotensin I was obtained from Bachem (U.S.A.). Hippuryl-His-Leu was obtained from Peptide Institute (Japan).

*Statistical analysis*: All data are presented as means ± standard error (SEM). Between-group comparisons at each time point were performed using a two-tailed Student's *t*-test. Within-group comparisons at each time point were performed using one-way ANOVA followed by the Bonferroni/Dunn test. Values of $p<0.05$ were considered to be statistically significant.

## RESULTS

*Clinical findings*: During the experiment, no hamsters were removed due to death, and none showed signs of such things as pneumonedema and pleural effusion.

Body weight and ventricular weight: The hamsters in the AS group had significantly higher body weights (g), ventricular weights (mg), and ventricular weight-to-body weight ratios than those in the Sham group at 20 weeks after banding ($p<0.01$) (Table 1).

*Echocardiography*: The detailed data for the AS and Sham groups are summarized in Table 2. In the Sham group, cardiac malformation and dysfunction were not shown, except for an increase in LVPWs/(LVIDs/2) at 20

Table 1. Body weight, ventricular weight, and ventricular weight-to-body weight ratio at 20 weeks after banding

| | n | BW (g) | VW (mg) | VW/BW(mg/g) |
|---|---|---|---|---|
| Sham | 16 | 117.2 ± 3.1 | 272.9 ± 3.0 | 2.34 ± 0.05 |
| AS | 19 | 129.3 ± 2.0** | 365.5 ± 16.9** | 2.83 ± 0.13** |

All data are presented as means ± standard error (SEM). BW: body weight; VW: ventricular weight; VW/BW: ventricular weight-to-body weight ratio. Sham: sham-operated group; AS: aortic stenosis group. Values are expressed as ** $p<0.01$ vs. Sham group.

Table 2. Echocardiographic data for preoperative and postoperative hamsters (10 and 20 weeks)

| | n | LVPWd/(LVIDd/2) | LVPWs/(LVIDs/2) | FS(%) |
|---|---|---|---|---|
| Sham | | | | |
| Preoperative | 6 | 0.532 ± 0.030 | 0.700 ± 0.043 | 23.4 ± 2.7 |
| Postoperative (10 weeks) | 6 | 0.553 ± 0.044 | 0.902 ± 0.049 | 27.4 ± 1.3 |
| Postoperative (20 weeks) | 6 | 0.540 ± 0.045 | 0.927 ± 0.081† | 26.6 ± 2.4 |
| AS | | | | |
| Preoperative | 6 | 0.538 ± 0.026 | 0.735 ± 0.021 | 21.7 ± 0.9 |
| Postoperative (10 weeks) | 6 | 0.773 ± 0.050††** | 1.358 ± 0.114††** | 30.98 ± 2.24† |
| Postoperative (20 weeks) | 6 | 0.543 ± 0.024‡‡ | 0.808 ± 0.068‡‡ | 25.86 ± 2.90 |

All data are presented as means ± standard error (SEM). LVPWd/(LVIDd/2): ratio of diastolic left ventricle wall thickness to internal radius; LVPWs/(LVIDs/2): ratio of systolic left ventricle wall thickness to internal radius; FS: left ventricular fractional shortening; Sham: sham-operated group; AS: aortic stenosis group. Values are expressed as †† $p<0.01$ vs. preoperative for the same group; † $p<0.05$ vs. preoperative for the same group; ‡‡ $p<0.01$ vs. postoperative (10 weeks) for the same group; ** $p<0.01$ vs. Sham group at the same time point.

weeks after banding compared with the preoperative level ($p<0.05$). In the AS group, LVPW/(LVID/2) at 10 weeks after banding was significantly higher ($p<0.01$) than the preoperative level in the AS group and the level at 10 weeks after banding in the Sham group. FS at 10 weeks after banding was significantly increased compared with the preoperative level in the AS group ($p<0.05$). The increase in LVPW/(LVID/2) between the preoperative measurement point and 10 weeks after banding in the AS group suggested concentric hypertrophy. Subsequently, LVPW/(LVID/2) at 20 weeks after banding was significantly decreased in the AS group compared with the level at 10 weeks after banding ($p<0.01$). The significant decrease in LVPW/(LVID/2) between 10 and 20 weeks after banding suggested that these hamsters were in the transition phase to heart failure. There was no difference in FS between 10 and 20 weeks after banding in the AS group.

*Left ventricular histological examination*: The AS group showed a significant increase in cardiac fibrosis (Fig. 1). In the Sham group, the collagen densities in the subendocardial and subepicardial regions of the left ventricle (2.274 ± 0.122 and 2.225 ± 0.178%, respectively) were significantly larger than that in the middle portion of the left venetricle (1.466 ± 0.078%, $p<0.01$). In the AS group, the collagen densities in the subendocardial, subepicardial, and middle portion of the left ventricle (4.273 ± 0.722, 3.470 ± 0.163, and 2.866 ± 0.322%, respectively) were significantly higher than those in the Sham group [2.274 ± 0.122 ($p<0.05$), 2.225 ± 0.178, and 1.466 ± 0.078% ($p<0.01$), respectively]. There were no differences in any region within the AS group.

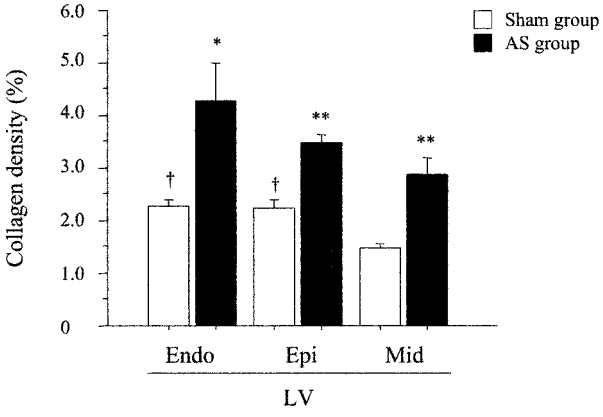


Fig. 1. Graph demonstrating the collagen density percent in the Sham (hollow bars) and AS groups (solid bars) at 20 weeks after banding. Endo: subendocardium of the left ventricle (LV); Epi: subepicardium of the LV; Mid: middle portion of the LV. Sham: sham-operated group; AS: aortic stenosis group. Values are expressed as † $p<0.01$ vs. Mid in the same group; ** $p<0.01$ vs. Sham group; * $p<0.05$ vs. Sham group.

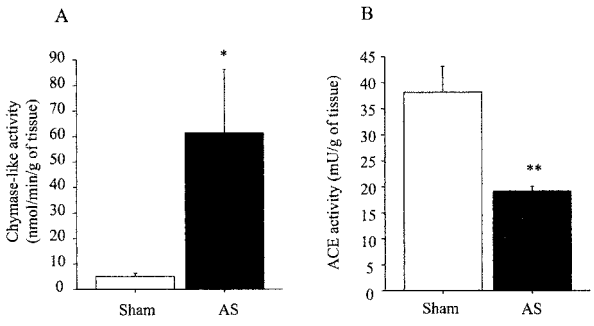


Fig. 2. Chymase-like activity (A) and angiotensin-converting enzyme (ACE) activity (B) in the Sham and AS groups at 20 weeks after banding. Sham: sham-operated group; AS: aortic stenosis group. Values are expressed as ** $p<0.01$ vs. Sham group; * $p<0.05$ vs. Sham group.

*ACE and chymase-like activities in the heart*: The AS group showed a significant increase in chymase-like activity (61.4 ± 24.7 nmol/min/g of tissue) compared with the Sham group (5.1 ± 1.3 nmol/min/g of tissue, $p < 0.05$, Fig. 2.A). In contrast, ACE activity in the AS group (18.9 ± 1.1 mU/g of tissue) was significantly lower compared with the Sham group (38.2 ± 5.0 mU/g of tissue, $p<0.01$, Fig. 2.B).

## DISCUSSION

Persistent pressure overload accelerates hypertrophic changes including fibrosis at the cardiac myocyte level, which is called cardiac remodeling [6]. Considering the clinical setting for canine heart with pressure overload, an experimental model induced by persistent pressure overload is necessary. Litwin *et al.* successfully produced heart failure in rats with persistent pressure overload-induced hypertrophy [15]. Species variation in chymase function has been found in the conversion of Ang I [11]. Human, dog, and hamster chymases convert Ang I to Ang II [1, 17], whereas rats not only convert Ang I to Ang II, but also degrade Ang II [20]. Thus, animal models other than the rat should be utilized to investigate the involvement of cardiac Ang II in the development of pressure overload-induced heart failure. In the present study, persistent pressure overload was established by ascending aortic stenosis, and the heart was evaluated using serial echocardiographic assessment and histopathological analysis. This model demonstrated the cardiac hypertrophy and cardiac fibrosis phases. For example, concentric hypertrophy of the left ventricle was present at 10 weeks after banding. In echocardiography, the findings were characterized by increased wall thickness, with reduced dimensions of the left ventricular cavity. At 20 weeks after banding, body weight, ventricular weight, and ventricular weight-to-body weight ratio were significantly increased and cardiac fibrosis appeared. The increase in ventricular weight and ventricular weight-to-body weight ratio in the AS group demonstrated cardiac hypertrophy, and congestive edema was considered the cause of the increase in body weight, although macroscopicical pneumonedema and pleural effusion were not exhibited in the AS group. The findings at 20 weeks after banding suggest that this stage was cardiac remodeling complicated by cardiac fibrosis, and this was a more advanced stage than cardiac hypertrophy [32]. Although cardiac remodeling is an important compensatory response to pressure overload, continued structural remodeling of the myocardium ultimately becomes maladaptive, leading to development of heart failure [6]. There is no data to indicate when the transition from possible adaptive to maladaptive remodeling occurs. Recently, it was reported that the myocardial matrix metalloproteinase mediated extracellular matrix degradation, resulting in thinning of the left ventricular wall thickness and left ventricular chamber dilatation, and contributing to heart failure progression [5]. Moreover, cardiac mast cells and chymase were associated with matrix metalloproteinase activation [25]. In the present model, the ratio of LVPW/(LVID/2) calculated as a concentric hypertrophy parameter revealed a significant reduction between 10 and 20 weeks after banding in echocardiography. This finding reveals that the stage of this model is the transition phase to the maladaptive stage.

The pathological stage of this model was earlier than that of the other hamster pressure overload model, the "two kidneys, one clip hamster model" [24], and the abdominal aortic stenosis hamster model [14]. These models of renal artery clipping and abdominal aortic stenosis lead to systemic blood hypertension, and then secondary left ventricular hypertrophy. In contrast, the pathology of ascending aortic banding is the same as that of hereditary supravalvular aortic stenosis. In this case, because pressure overload to the left ventricle occurred without systemic blood hypertension, the load against the left ventricle was direct. Early appearance of cardiac fibrosis leads to shortening of the experimental period. The diameter of the ascending aorta is thick enough to band, so the constant stricture ratio obtained was greater than that for the abdominal aorta or kidney artery, and thus a stabilized model could be prepared. Although the high stricture ratio increased the risk of acute pneumonedema, the present method produced a model with a 100% survival rate.

Moreover, the cardiac ACE and chymase-like activities were measured to establish the involvement of these enzymes in the hamster heart with pressure overload. In dogs, detailed analyses have revealed that the RAS existing in cardiac tissue has a crucial role in the development of hypertrophy [2]. Cardiac ACE plays a role not only in generating Ang II, which provokes cardiac hypertrophy and fibrosis through Ang II type 1 (AT1) receptor signaling [4, 12, 23], but also in increasing cardiac fibrosis by catalyzing the degradation of bradykinin into inactive metabolites [3, 9, 26, 30]. Similarly, cardiac chymase plays a role not only in converting Ang I to Ang II [31], but also in promoting direct collagen production [28, 29]. In a report on the left ventricle from dogs with pressure overload due to ascending aortic stenosis, the cardiac ACE and chymase-like activities were

increased [21]. In one hamster pressure overload model, the "two kidneys, one clip hamster model", cardiac chymase activity significantly increased after clipping, while the cardiac ACE activity remained unchanged after clipping [24]. In the hamsters of the other pressure overload model, abdominal aortic stenosis, the cardiac ACE and chymase activities significantly increased, however, the level of chymase activity was higher than that of the ACE activity during the development of cardiac hypertrophy [14]. These results suggest that the increase in Ang II levels via chymase plays an important role in the development of cardiac hypertrophy and fibrosis. Cardiac chymase-like activity in the present model was significantly higher at 20 weeks after banding, whereas cardiac ACE activity was significantly lower. This suggests that persistent pressure overload induced the increase in chymase-like activity, and that the chymase-like activity was associated with cardiac remodeling through Ang II formation. There is currently no data indicating depression of cardiac ACE activity in hamsters with pressure overload. However, previous report revealed that angiotensinogen produced by cultured neonatal rat cardiac fibroblasts is under negative feedback control of Ang II [8], and the increase in Ang II via chymase-like activity is considered to have caused compensatory depression of ACE activity by a specific mechanism. Other studies have produced similar findings indicating that cardiac chymase-like activity was increased by more than ACE activity in the heart with pressure overload in hamsters [24] and in heart failure in dogs [7], and they have also indicated that chymase-like activity might be involved in cardiac tissue remodeling during the chronic stage. Thus, the results in hamster may supplement the available information for investigative study of the relationship between cardiac RAS and cardiac remodeling in dogs with heart failure.

As the Ang II-forming pathway and the effect of chymase of the hamster is similar to that of dogs, the evidence produced from the present model is more reliable than that in the rat model when demonstrating Ang II formation through cardiac chymase and cardiac remodeling mediated by cardiac Ang II produced by chymase. The present model is expected to be considered appropriate for studies investigating the effect of Ang II and chymase in the canine heart with chronic pressure overload.

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REFERENCES

1. Balcells, E., Meng, Q. C., Johnson, W. H., Jr., Oparil, S. and Dell'Italia, L. J. 1997. Angiotensin II formation from ACE and chymase in human and animal hearts: methods and species considerations. *Am. J. Physiol.* **273**: H1769–1774.
2. Barlucchi, L., Leri, A., Dostal, D. E., Fiordaliso, F., Tada, H., Hintze, T. H., Kajstura, J., Nadal-Ginard, B. and Anversa, P. 2001. Canine ventricular myocytes possess a renin-angiotensin system that is upregulated with heart failure. *Circ. Res.* **88**: 298–304.
3. Busatto, V. C., Cicilini, M. A. and Mill, J. G. 1997. Increased angiotensin-converting enzyme activity in the left ventricle after infarction. *Braz. J. Med. Biol. Res.* **30**: 679–687.
4. Campbell, S. E. and Katwa, L. C. 1997. Angiotensin II stimulated expression of transforming growth factor-beta1 in cardiac fibroblasts and myofibroblasts. *J. Mol. Cell. Cardiol.* **29**: 1947–1958.
5. Chancey, A. L., Brower, G. L., Peterson, J. T. and Janicki, J. S. 2002. Effects of matrix metalloproteinase inhibition on ventricular remodeling due to volume overload. *Circulation* **105**: 1983–1988.
6. Cohn, J. N., Ferrari, R. and Sharpe, N. 2000. Cardiac remodeling--concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *J. Am. Coll. Cardiol.* **35**: 569–582.
7. Dell'Italia, L. J., Meng, Q. C., Balcells, E., Straeter-Knowlen, I. M., Hankes, G. H., Dillon, R., Cartee, R. E., Orr, R., Bishop, S. P., Oparil, S. *et al.* 1995. Increased ACE and chymase-like activity in cardiac tissue of dogs with chronic mitral regurgitation. *Am. J. Physiol.* **269**: H2065–2073.
8. Dostal, D. E., Booz, G. W. and Baker, K. M. 2000. Regulation of angiotensinogen gene expression and protein in neonatal rat cardiac fibroblasts by glucocorticoid and beta-adrenergic stimulation. *Basic Res. Cardiol.* **95**: 485–490.
9. Fujii, M., Wada, A., Ohnishi, M., Tsutamoto, T., Matsumoto, T., Yamamoto, T., Takayama, T., Dohke, T., Isono, T., Eguchi, Y. and Horie, M. 2004. Endogenous bradykinin suppresses myocardial fibrosis through the cardiac-generated endothelin system under chronic angiotensin-converting enzyme inhibition in heart failure. *J. Cardiovasc. Pharmacol.* **44**: S346–S349.
10. Garg, R. and Yusuf, S. 1995. Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure. Collaborative Group on ACE Inhibitor Trials. *Jama.* **273**: 1450–1456.
11. Hollenberg, N. K. 2000. Implications of species difference for clinical investigation: studies on the renin-angiotensin system. *Hypertension* **35**: 150–154.
12. Ju, H. and Dixon, I. M. 1996. Effect of angiotensin II on myocardial collagen gene expression. *Mol. Cell. Biochem.* **163–164**: 231–237.
13. Konstam, M. A., Rousseau, M. F., Kronenberg, M. W., Udelson, J. E., Melin, J., Stewart, D., Dolan, N., Edens, T. R., Ahn, S., Kinan, D. *et al.* 1992. Effects of the angiotensin converting enzyme inhibitor enalapril on the long-term progression of left ventricular dysfunction in patients with heart failure. SOLVD Investigators. *Circulation* **86**: 431–438.
14. Li, P., Chen, P. M., Wang, S. W. and Chen, L. Y. 2002. Time-dependent expression of chymase and angiotensin converting enzyme in the hamster heart under pressure overload. *Hypertens. Res.* **25**: 757–762.
15. Litwin, S. E., Katz, S. E., Weinberg, E. O., Lorell, B. H., Aurigemma, G. P. and Douglas, P. S. 1995. Serial echocardiographic-Doppler assessment of left ventricular geometry and function in rats with pressure-overload hypertrophy. Chronic angiotensin-converting enzyme inhibition attenuates the transition to heart failure. *Circulation* **91**: 2642–2654.
16. Mitsuhashi, S., Saito, N., Watano, K., Igarashi, K., Tagami, S., Shima, H. and Kikuchi, K. 2003. Defect of delta-sarcoglycan gene is responsible for development of dilated cardiomyopathy

of a novel hamster strain, J2N-k: calcineurin/PP2B activity in the heart of J2N-k hamster. *J. Biochem.* **134**: 269–276.
17. Nishimura, H., Buikema, H., Baltatu, O., Ganten, D. and Urata, H. 1998. Functional evidence for alternative ANG II-forming pathways in hamster cardiovascular system. *Am. J. Physiol.* **275**: H1307–1312.
18. Orito, K., Yamane, T., Kanai, T., Fujii, Y., Wakao, Y. and Matsuda, H. 2004. Time course sequences of angiotensin converting enzyme and chymase-like activities during development of right ventricular hypertrophy induced by pulmonary artery constriction in dogs. *Life Sci.* **75**: 1135–1145.
19. Sadoshima, J., Xu Y., Slayter, H. S. and Izumo, S. 1993. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes *in vitro*. *Cell* **75**: 977–984.
20. Sanker, S., Chandrasekharan, U. M., Wilk, D., Glynias, M. J., Karnik, S. S. and Husain, A. 1997. Distinct multisite synergistic interactions determine substrate specificities of human chymase and rat chymase-1 for angiotensin II formation and degradation. *J. Biol. Chem.* **272**: 2963–2968.
21. Schultz, D., Su, X., Wei, C. C., Bishop, S. P., Powell, P., Hankes, G. H., Dillon, A. R., Rynders, P., Spinale, F. G., Walcott, G., Ideker, R. and Dell'Italia, L. J. 2002. Downregulation of ANG II receptor is associated with compensated pressure-overload hypertrophy in the young dog. *Am. J. Physiol. Heart Circ. Physiol.* **282**: H749–756.
22. Schunkert, H., Dzau, V. J., Tang, S. S., Hirsch, A. T., Apstein, C. S. and Lorell, B. H. 1990. Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy. Effects on coronary resistance, contractility, and relaxation. *J. Clin. Invest.* **86**: 1913–1920.
23. Sharma, H. S., van Heugten, H. A., Goedbloed, M. A., Verdouw, P. D. and Lamers, J. M. 1994. Angiotensin II induced expression of transcription factors precedes increase in transforming growth factor-beta 1 mRNA in neonatal cardiac fibroblasts. *Biochem. Biophys. Res. Commun.* **205**: 105–112.
24. Shiota, N., Jin, D., Takai, S., Kawamura, T., Koyama, M., Nakamura, N. and Miyazaki, M. 1997. Chymase is activated in the hamster heart following ventricular fibrosis during the chronic stage of hypertension. *FEBS. Lett.* **406**: 301–304.
25. Stewart, J. A., Jr., Wei, C. C., Brower, G. L., Rynders, P. E., Hankes, G. H., Dillon, A. R., Lucchesi, P. A., Janicki, J. S. and Dell'Italia, L. J. 2003. Cardiac mast cell- and chymase-mediated matrix metalloproteinase activity and left ventricular remodeling in mitral regurgitation in the dog. *J. Mol. Cell. Cardiol.* **35**: 311–319.
26. Sun, Y., Ratajska, A. and Weber, K. T. 1995. Bradykinin receptor and tissue ACE binding in myocardial fibrosis: response to chronic angiotensin II or aldosterone administration in rats. *J. Mol. Cell. Cardiol.* **27**: 813–822.
27. Swedberg, K., Pfeffer, M., Granger, C., Held, P., McMurray, J., Ohlin, G., Olofsson, B., Ostergren, J. and Yusuf, S. 1999. Candesartan in heart failure--assessment of reduction in mortality and morbidity (CHARM): rationale and design. Charm-Programme Investigators. *J. Card. Fail.* **5**: 276–282.
28. Taipale, J., Lohi, J., Saarinen, J., Kovanen, P. T. and Keski-Oja, J. 1995. Human mast cell chymase and leukocyte elastase release latent transforming growth factor-beta 1 from the extracellular matrix of cultured human epithelial and endothelial cells. *J. Biol. Chem.* **270**: 4689–4696.
29. Takai, S., Jin, D., Sakaguchi, M., Katayama, S., Muramatsu, M., Matsumura, E., Kim, S. and Miyazaki, M. 2003. A novel chymase inhibitor, 4-[1-([bis-(4-methyl-phenyl)-methyl]-carbamoyl)3-(2-ethoxy-benzyl)-4-oxo-a zetidine-2-yloxy]-benzoic acid (BCEAB), suppressed cardiac fibrosis in cardiomyopathic hamsters. *J. Pharmacol. Exp. Ther.* **305**: 17–23.
30. Tanaka, Y., Nagai, M., Date, T., Okada, T., Abe, Y., Seki, S., Taniguchi, M., Taniguchi, I. and Mochizuki, S. 2004. Effects of bradykinin on cardiovascular remodeling in renovascular hypertensive rats. *Hypertens. Res.* **27**: 865–875.
31. Urata, H., Kinoshita, A., Misono, K. S., Bumpus, F. M. and Husain, A. 1990. Identification of a highly specific chymase as the major angiotensin II-forming enzyme in the human heart. *J. Biol. Chem.* **265**: 22348–22357.
32. Weber, K. T. 2000. Targeting pathological remodeling: concepts of cardioprotection and reparation. *Circulation* **102**: 1342–1345.
33. Wei, C. C., Meng ,Q. C., Palmer, R., Hageman, G. R., Durand, J., Bradley, W. E., Farrell, D. M., Hankes, G. H., Oparil, S. and Dell'Italia, L. J. 1999. Evidence for angiotensin-converting enzyme- and chymase-mediated angiotensin II formation in the interstitial fluid space of the dog heart *in vivo*. *Circulation* 99: 2583–2589.
34. Weinberg, E. O., Lee, M. A., Weigner, M., Lindpaintner, K., Bishop, S. P., Benedict, C. R., Ho, K. K., Douglas, P. S., Chafizadeh, E. and Lorell, B. H. 1997. Angiotensin AT1 receptor inhibition. Effects on hypertrophic remodeling and ACE expression in rats with pressure-overload hypertrophy due to ascending aortic stenosis. *Circulation* **95**: 1592–1600.
35. Zhang, X., Dostal, D. E., Reiss, K., Cheng, W., Kajstura, J., Li, P., Huang, H., Sonnenblick, E. H., Meggs, L. G., Baker, K. M. and *et al.* 1995. Identification and activation of autocrine renin-angiotensin system in adult ventricular myocytes. *Am. J. Physiol*. **269**: H1791–1802.