Increase of Angiotensin Converting Enzyme Gene Expression in the Hypertensive Aorta

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To investigate the possible role of vascular angiotensin converting enzyme (ACE) in the development and maintenance of hypertension, we examined aortic ACE messenger RNA (mRNA) levels in two-kidney, one clip (2K1C) hypertensive rats. The blood pressure was increased remarkably at 4 weeks (early stage) after clipping and remained elevated at 12 weeks (chronic stage). The aorta ACE mRNA levels were significantly elevated in both early and chronic stages concurrently with the increases in aortic ACE activity and blood pressure. The plasma renin activity rose markedly at 4 weeks, but returned to the normal level at 12 weeks. Neither ACE activity in the lung and plasma, nor ACE mRNA level in the lung was altered at either stage. The aorta and liver angiotensinogen mRNA levels and renal renin mRNA level were increased at 4 weeks but decreased at 12 weeks. These results indicate that the acceleration of all components in the renin-angiotensin system may contribute to the development of 2K1C hypertension in the early stage. In the chronic stage, the increased vascular ACE induced by the elevated ACE mRNA levels in the aorta may play the primary role in the acceleration of local angiotensin II formation and thus may sustain the hypertension. (Hypertension 1992;20:168-174)

KEY WORDS • kininase II • RNA, messenger • aorta • hypertension, renal • angiotensinogen • renin

Although numerous studies have suggested the existence of intrinsic renin-angiotensin system (RAS) in multiple tissues and organs,1,2 little has been clarified for its pathophysiological significance. In particular, little attention has been focused on the pathophysiological roles of vascular angiotensin converting enzyme (ACE). The lack of interest may be due to the fact that the ACE is distributed in abundance throughout vascular systems, and this makes it a quite unlikely candidate for regulating the blood pressure. We previously have proposed the relevance of the vascular ACE to the pathogenesis of hypertension; these studies used various types of hypertensive models: two-kidney, one clip (2K1C) rats3,4 and dogs5,6; one-kidney, one clip (1K1C) rats7; and spontaneously hypertensive rats (SHR).8 In the chronic hypertensive stages of these models, the circulating RAS was unlikely to be responsible for the maintenance of high blood pressure because the plasma renin activity (PRA) and plasma ACE activity were not high. In contrast, the vascular ACE activity was consistently high irrespective of the different pathogenic backgrounds. This may imply that the increased ACE in the vascular tissues sustains the hypertension via an enhanced generation of angiotensin II (Ang II). This idea is also supported by the following findings: 1) in any hypertensive models used previously, depressor responses to ACE inhibitors as well as Ang II antagonists were linearly related with the increase in vascular ACE activity3-6; 2) the development of hypertension in the SHR coincided with an increase in aortic ACE activity.8 This suggests that the vascular ACE may be involved in the pathogenesis of hypertension to a much greater extent than has generally been considered.

With the use of molecular biological techniques, studies have been performed to investigate the molecular mechanisms that regulate the biosynthesis of renin9 and angiotensinogen.10 Through these studies, the existence of tissue RAS was recognized, and its role has begun to be investigated intensely. In addition, the primary structures of endothelial and testicular ACE from human,11-13 rabbit,14,15 and mouse16 have recently been determined from the complementary DNA (cDNA) clones. Although the structural and enzymological aspects of the ACE molecule were studied extensively with the cDNA clones,17-20 few investigators have thus far used those clones to explore the regulatory mechanism of ACE biosynthesis. Furthermore, very few studies have investigated the molecular mechanism of ACE biosynthesis in pathophysiological states. The use of an ACE cDNA probe has enabled us to detect ACE messenger RNA (mRNA) and has provided us with direct evidence of how ACE protein is expressed in specific tissues. We report a study examining the changes in ACE mRNA levels during the early and chronic stages of 2K1C hypertensive rats. Concurrently, the levels of angiotensinogen mRNA and renin mRNA were determined. The present study provides important
Evidence that supports the role of vascular ACE in the state of hypertension.

**Methods**

Six-week-old male Wistar rats (weighing 145-150 g) were purchased from Clea Japan, Tokyo, and were divided into two groups: a 2K1C renal hypertensive group and a sham-operated control group. The rats were fed with regular rat chow (Clea Japan), given free access to tap water, and housed under the same conditions throughout the experimental period. The experimental procedures for animals were in accordance with institutional guidelines.

**Experimental Protocol and Tissue Processing**

The 2K1C hypertension was induced as reported previously. The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and their left renal arteries were narrowed by a silver clip (0.2 mm i.d.) while the right renal arteries were kept intact. The control rats were subjected to a sham operation in which the same type of clip was placed adjacent to the left renal artery. The animals were randomly allocated to either the early group (4 weeks after clipping) or the chronic group (12 weeks after clipping), and the mean blood pressure was measured. To minimize the effect of anesthesia and surgical damage, a polyethylene catheter (PE-50, Clay Adams, Parsippany, N.J.) was placed in the femoral artery and exteriorized through a subcutaneous tunnel at the back of the neck 1 day before the study. The catheter was filled with heparinized saline and a small air bubble to allow for pulsation, which prevented the blood from clotting in the tube. Animals were allowed to recover for 24 hours before initiation of the experimental procedures. On the day of the experiment, the catheter was connected to a pressure transducer (Nihon Kohden Kogyo, Tokyo), and mean blood pressure values were measured under conscious and nonrestricted conditions. Then, blood samples were obtained through the catheter for the determination of PRA and plasma ACE activities. The rats were then killed by decapitation, and their left renal arteries were kept intact. The lung, aorta, liver, and kidney were collected for determination of the tissue ACE activities and mRNA levels of ACE, angiotensinogen, and renin. The whole aorta was dissected from the branching point of the left subclavian artery down to the bifurcation of the iliac artery, and the periaortic fat was carefully separated into the hybridization buffer containing the phosphorus-32 labeled probe. After this procedure, the blotted nylon membranes were washed twice at room temperature in a solution containing 2× SSC, 0.1% SDS at 50°C for 10 minutes and then twice in a solution containing 0.5× SSC, 0.1% SDS at 65°C for 30 minutes. Autoradiography was performed at -80°C using RX-film (Fuji Film Co, Tokyo) with two intensifying screens (Du Pont Co., Wilmington, Del.).
Quantification of Specific Messenger RNA Levels

Quantification of specific mRNA levels was performed by using the modified method of Heinrich et al.\textsuperscript{26} Three serial amounts of the total RNA taken from individual rats were used, and the autoradiograms were scanned down with a densitometer (CS-9000, Dual-Wavelength Flying-Spot Scanner, Shimadzu, Kyoto, Japan). The integrated areas of the hybrid images (arbitrary units) were plotted against the three serial quantities of the total RNA, and the regression lines were assigned only when the \( r \) values were higher than 0.99. The slope of the linear regression line represented the relative level of specific mRNA. The relative difference in specific mRNA levels between the 2K1C and the control groups was calculated from the ratio of mean slope values. The comparison of the mRNA levels of each tissue was made by a single autoradiogram. Three serial amounts of the RNA standards from the lung and testis of a single sham-operated rat (4 weeks after surgery) were run on every Northern blot for ACE mRNA; these standards were used to normalize the interblot variance. For the same reason, the liver RNA and the kidney RNA from the same single rat as above were run on every Northern blot for angiotensinogen mRNA and renin mRNA, respectively.

Statistical Analyses

All numbers shown in the text are expressed as mean±SEM. Significant differences between the means of different groups were evaluated by the Student's t test for unpaired data and by the modified t test for multiple comparison (Tukey's method) after a one-way analysis of variance, where appropriate.

Results

The mean blood pressure of 2K1C rats at 4 weeks after clipping was higher (212±3 mm Hg, \( n=18 \)) than that of age-matched sham-operated control rats (119±2 mm Hg, \( n=10 \)) and remained high at 12 weeks after clipping (217±4 mm Hg, \( n=16 \) versus 121±1 mm Hg, \( n=10 \) for age-matched controls). As shown in Table 1, the body weight of 2K1C rats was less than that of age-matched controls at either the early (4 weeks after clipping) or the chronic stage (12 weeks after). The weight of the clipped left kidney in the early stage was less than that of the contralateral kidney, but the weights of the left and right kidneys of 2K1C rats were similar and comparable to those of the sham-operated rats in the chronic stage (Table 1).

PRA in 2K1C rats was dramatically increased in the early stage (166.6±50.8 ng Ang I/ml · hr\(^{-1}\)) and was significantly higher than that in sham-operated rats (3.7±0.8 ng Ang I/ml · hr\(^{-1}\)). The PRA was decreased to 29.4±5.8 ng Ang I/ml · hr\(^{-1}\) in the chronic stage as compared with that in the acute stage, yet this value was higher than that of the age-matched controls (2.5±0.5 ng Ang I/ml · hr\(^{-1}\)). The plasma ACE activity in 2K1C rats was not significantly different from the age-matched sham-operated rats either in the early stage (83.3±6.8 versus 75.0±2.7 milliunits/ml) or in the chronic stage (84.5±11.8 versus 69.0±3.9 milliunits/ml).

Figure 1 shows a typical example of Northern blot analysis of ACE mRNA in the aorta from a 2K1C hypertensive rat at 4 weeks after clipping as well as one from an age-matched sham-operated rat. This figure indicates that aortic ACE mRNA is detected as a single band and identical to pulmonary ACE mRNA. In the

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Representative Southern blot analysis of rat aortic angiotensin converting enzyme (ACE) messenger RNA (mRNA) in two-kidney, one clip hypertensive rats (2K1C) and sham-operated control rats (Sham) at 4 weeks after operation and pulmonary and testicular ACE mRNA in Sham at 4 weeks after operation. The blot membrane was hybridized with an oligolabeled human endothelial ACE complementary DNA (cDNA) probe. Arrowheads at 28S and 18S indicate respective positions of ribosomal RNA transferred to nylon membrane. Amount of total RNA applied into each lane is indicated thereunder.
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FIGURE 2. Bar graphs show aortic angiotensin converting enzyme (ACE) messenger RNA (mRNA) level (panel A) and aortic ACE activity (panel B) in two-kidney, one clip hypertensive rats (2K1C) and sham-operated control rats (Sham) at 4 and 12 weeks after operation. Vertical bars represent SEM. Number of rats is shown in parentheses. Specific mRNA level is expressed in arbitrary units per microgram weight of total RNA (slope). **p<0.01, ***p<0.001 vs. respective controls.

early stage, the hybridized signal in the aorta from the 2K1C rat was increased compared with that from the sham-operated rat. Figure 2A shows the densitometric analysis of aortic ACE mRNA levels in the early and chronic stages. The level of aortic ACE mRNA from 2K1C rats exhibited a 2.6-fold increase compared with that from age-matched sham-operated rats (p<0.01) in the early stage. In the chronic stage, this increase was 1.9-fold (p<0.01). The aortic ACE activity of 2K1C rats was significantly higher than that of age-matched controls both in the early stage (34.1±1.9 versus 13.9±0.8 milliunits/mg protein, p<0.001) and in the chronic stage (37.3±2.0 versus 15.4±1.0 milliunits/mg protein, p<0.001, Figure 2B). No significant changes occurred in pulmonary ACE mRNA level or ACE activity in either the early or the chronic stage (Figure 3).

A representative Northern blot of aortic angiotensinogen mRNA in 2K1C rats and sham-operated rats in the early and chronic stages is shown in Figure 4, which indicates the increase in angiotensinogen mRNA signals from 2K1C rats in the early stage. In contrast, aortic angiotensinogen mRNA signals were similar for both experimental groups in the chronic stage. Figure 5A shows densitometric analysis of aortic angiotensinogen mRNA levels. In the early stage, the aortic angiotensinogen mRNA level in 2K1C rats exhibited a 4.9-fold increase compared with that in age-matched controls (p<0.001), whereas in the chronic stage, the level in 2K1C rats decreased significantly (p<0.001) toward a value that was similar to that of sham-operated rats. Liver angiotensinogen mRNA levels were also analyzed, and the results were similar (Figure 5B). The liver angiotensinogen mRNA level in 2K1C rats in the early stage was increased 2.4-fold compared with that in age-matched sham-operated rats (p<0.001). In the chronic stage, however, the liver angiotensinogen mRNA level in 2K1C rats decreased significantly (p<0.001), although it was still higher than that of age-matched controls.

Figure 6 shows the changes in renal renin mRNA levels. In the early stage, the renal renin mRNA level was 12-fold higher in the clipped left kidney of 2K1C rats compared with the left kidney of age-matched sham-operated rats (p<0.001). In the chronic stage, the level in the clipped left kidney was significantly decreased (p<0.01), yet it was higher than that of sham-operated controls. In the aorta, lung, liver, and kidney, no differences were detected between the nonmuscle β-actin mRNA levels of the two experimental groups in either the early or chronic stage (data not shown).

Discussion

With the use of Northern blot analyses, we showed for the first time that the ACE mRNA was locally expressed in the aorta and that the ACE mRNA levels can be altered in a pathological state. The increase in the ACE
mRNA correlated not only with the increase in aortic ACE activity but also with the rise in blood pressure at both early and chronic stages of 2K1C hypertension. The increased ACE activity can be attributed to an exaggerated ACE biosynthesis induced by the elevated aortic ACE mRNA level; this rise in the mRNA level may be due to the alterations in the transcription rate of ACE gene or in the stability of ACE mRNA. It has been generally believed that the RAS contributes little for the maintenance of 2K1C hypertension in the chronic stage, although it plays a major role for the development of hypertension in the early stage. However, we postulated that vascular ACE plays a role in maintaining hypertension of 2K1C and other experimental models. The present results have provided additional evidence for the pathogenetic importance of vascular ACE in the maintenance as well as the development of 2K1C hypertension from the aspect of ACE gene expression.

The present results have also raised further questions of how the ACE gene expression is induced selectively in the hypertensive vascular tissues and how the hypertension and the ACE expression affect each other. Our hypothesis to the first question is that the hypertension per se could induce ACE in the tissues that are exposed to the high wall tension. The cardiac ACE was induced in the hypertrophied ventricle from rats with experimental heart failure and with aortic stenosis. In the developmental phase of SHR, an increase in vascular ACE activity coincided with an elevation in systemic blood pressure. These findings suggest the presence of an interaction between the blood pressure and the induction of ACE gene expression. Moreover, our preliminary study demonstrated that a mechanical decompression of intra-aortic pressure in 2K1C rats and SHR at their chronic hypertensive stages resulted in a decrease in aortic ACE activity back to the prehypertensive baseline (unpublished data from our laboratory). This result would indicate that the ACE induction is the consequence of high blood pressure stress on the vascular wall.

Although high blood pressure itself can induce ACE gene expression, the induction of vascular ACE is not merely the consequence of hypertension. The exaggerated ACE biosynthesis and the consequent increase in Ang II formation in the vascular wall should also be the cause of sustained hypertension. Such a vicious cycle between hypertension and induction of ACE might be constructed in the vascular wall, particularly in the chronic phase of hypertension. The increased vascular ACE should play a pathogenetic role in a key step of the cycle. The potential existence of such a positive feedback mechanism would provide an answer to the second question.

Although the precise molecular mechanism that links hypertension and induction of ACE expression remains unknown, some examples of the pressure-induced gene expression have been reported. Hypertension can increase the aortic mRNA levels for platelet-derived growth factor \( \beta \) receptor and for fibronectin. Other investigators have demonstrated that fibroblast growth factor, known to stimulate the growth of vascular endothelial cells, could induce ACE activity in these cells. These findings suggest that various growth factors or vasoactive agents may be involved in the hypertension-induced ACE gene expression in the vascular wall.

**Figure 4.** Representative Northern blot analysis of rat aortic angiotensinogen messenger RNA in two-kidney, one clip hypertensive rats (2K1C) and sham-operated control rats (Sham) at 4 and 12 weeks after operation. The blot membrane was hybridized with an oligolabeled rat angiotensinogen complementary DNA probe. The arrowhead at 18S indicates the position of ribosomal RNA transferred to nylon membrane. The amount of total RNA applied is indicated under each lane.

**Figure 5.** Bar graphs show aortic angiotensinogen messenger RNA (mRNA) level (panel A) and liver angiotensinogen mRNA level (panel B) in two-kidney, one clip hypertensive rats (2K1C) and sham-operated control rats (Sham) at 4 and 12 weeks after operation. Vertical bars represent SEM. Number of rats used is shown in parentheses. Specific mRNA level is expressed in arbitrary units per microgram weight of total RNA (slope). *\( p<0.05 \), **\( p<0.01 \), ***\( p<0.001 \) vs. respective controls.
In contrast with the aorta, ACE activity in the lung and plasma or ACE mRNA level in the lung did not change in 2K1C rats in either stage. Moreover, we found that lung and plasma ACE activities in SHR were consistently lower than those in age-matched Wistar-Kyoto rats irrespective of blood pressure levels. The reason for such differences in aorta and lung is unknown. However, these findings suggest that ACE in the lung and plasma does not affect directly the elevation of systemic blood pressure. It is also suggested that ACE may play a different role, and its expression is regulated in a different fashion in the lung that is exposed to low perfusion pressure but not to high systemic blood pressure.

Angiotensinogen mRNA levels in the aorta were significantly increased in 2K1C rats at 4 weeks, but returned to the normal level at 12 weeks after clipping. The amounts of angiotensinogen expressed also showed an increase in the atria and ventricles of rats in the early phase after abdominal aorta constriction and experimental myocardial infarction, and in the smooth muscle cells from aorta of rats after 5 days of sodium restriction. But angiotensinogen expression was normalized 25 days later in the chronic heart failure state of experimental myocardial infarction. These findings suggest that angiotensinogen may contribute to the hemodynamic changes only in the acute phase.

Renal renin mRNA levels and the PRA were increased in the early stage of 2K1C rats, but were significantly decreased toward the normal level in the chronic stage. This indicates that the increase in circulating renin of renal origin is responsible for the development of hypertension in the early stage of 2K1C rats, but the circulating renin may no longer be a determinant for the maintenance of hypertension in the chronic stage. Although we could not measure the aortic renin mRNA level quantitatively because of its very low level, our previous experiment has shown that vascular renin activity was elevated during the early stage but returned to the normal level in the chronic stage of 2K1C hypertension. Thus, it is unlikely that the Ang I formation due to increase of renin level in the vascular wall may be responsible for maintaining the hypertension.

All the above results suggest that the ACE plays a more important role in generating Ang II than the renin or the angiotensinogen does, and thus the regulation of ACE gene expression should be a key issue in hypertension. Potential relevance of the ACE gene to hypertension is also supported by findings reported lately. With use of a linkage analysis technique with rats crossed between the stroke-prone SHR and the Wistar-Kyoto rat, the BP1/SP-1 or Bpl gene that regulates the blood pressure has been found to be closely linked to the ACE gene. In these studies, the ACE gene is proposed as a candidate gene for the pathogenesis of hypertension. In future, the investigation of the molecular mechanisms underlying the tissue-specific regulation of ACE gene expression is probably the most wanted step for completing the study on the pathogenesis of hypertension.

In conclusion, we have demonstrated that the exaggerated ACE biosynthesis in the vascular wall induced by the overexpression of the ACE gene plays a pivotal role in the development and maintenance of hypertension in 2K1C rats and that the ACE of vascular origin is a rate-limiting factor for the local Ang II generation, particularly in the chronic stage of hypertension.

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References


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