# Angiotensin Converting Enzyme Activity in Compensatory Renal Hypertrophy

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## Abstract

Serum and tissue (kidney's) angiotensin-converting enzyme (ACE) activity has been examined in Wistar rats (10 males and 10 females), seven days after unilateral nephrectomy. Renal hypertrophy was determined by measurement of kidney absolute mass. Serum and tissue ACE activity was determined by spectrophotometric method using hippuryl-l-histidyl-l-leucine (Hip-His-Leu) as a substrate. The ACE serum activity was expressed in units that correspond to 1 nmol of hippuric acid released by enzymatic hydrolysis of Hip-His-Leu substrate per minute/ml serum. The ACE tissue activity was expressed in units that correspond to 1 nmol of hippuric acid released by enzymatic hydrolysis of Hip-His-Leu substrate per minute/mg protein or mg kidney's tissue. The ACE serum activity significantly increased ($p<0,05$) seven days after unilateral nephrectomy. The ACE tissue activity, expressed in units that corresponds to 1 nmol of hippuric acid released by hydrolysis of Hip-His-Leu substrate per minute/mg protein, was higher seven days after unilateral nephrectomy then in kidney control, but the difference was not significant compared to the values determined in kidney control. The ACE tissue activity, expressed in units that correspond to 1 nmol of hippuric acid released by hydrolysis of Hip-His-Leu substrate per minute/mg tissue, was increased seven days after unilateral nephrectomy, which is statistically significant compared to the activity of the same enzyme in kidney control ($p<0,01$). The results indicate that ACE, probably has an important role in development of adaptive compensatory mechanisms after unilateral nephrectomy.

KEY WORDS: angiotensin converting enzyme, unilateral nephrectomy,
compensatory renal hypertrophy

## Introduction

Angiotensin converting enzyme (ACE; EC 3.4.15.1.) is a dipeptidyl carboxypeptidase that removes the C-terminal dipeptide from angiotensin I to produce the potent vasoconstrictor and growth-promoting substance angiotensin II. ACE also inactivates the vasodepressor bradykinin by the sequential removal of two C- terminal dipeptides (1). In addition to circulating (systemic) renin angiotensin system (RAS), there are local (tissue) RAS in heart, brain, kidneys, pancreas, and other tissues, that actions independent from circulating (2). The intrarenal renin angiotensin system has important role in regulation blood flow through kidneys and effects

on glomerular filtration rate and renal tubular function. This local RAS probably plays an important role as a regulator of renal sympathetic activity, modifies mesangial cell function, acts as a renal growth promoter, maintains endothelial cell function, and may be an important inflammatory mediator in the kidney (3). Local's synthesis angiotensin II in renal interstitial tissue and in the tubular epithelial cells regulates transport through cell's membranes of the proximal and distal tubules, pH urine and takes part to keep homeostasis water in the body. Wolf et al. (4) showed that angiotensin II is a renal growth factor. They demonstrated that angiotensin II, in a dose dependent manner, induces cellular enlargement and stimulates protein synthesis, leading to an increase in total protein contents (5). The loss of renal mass or unilateral nephrectomy is associated with functional and morphological adaptations in the remaining tissue. The most prominent response after unilateral nephrectomy is an increase in size and weight of the remaining kidney. This adaptive process is associated with hyperfiltration and increased transport capacity of the nephron epithelia (6). Amorena et al. (7) established that activity of renin angiotensin system increase in compensatory renal hypertrophy in aim to keep homeostasis water in organism. Other hand, Valentin et al. (8) did not show changes in some components of the renin angiotensin system in compensatory renal hypertrophy. The investigations of ACE activity in compensatory renal hypertrophy are very short. Michel et al. (9) examined the effects of dietary protein and uninephrectomy on renal ACE activity, showed that ACE activity in serum does not change in rats that were on standard protein diet after unilateral nephrectomy. For difference of that, ACE activity in the renal cortex and brush border increased statistical significantly 8 days after surgery in rats that were on standard protein diet after unilateral nephrectomy. Correlation between serum and tissue ACE activity in compensatory renal hypertrophy was not studied. The aim of this study was to investigate serum and tissue ACE activity in rats seven days after unilateral nephrectomy and to examine correlation between serum and tissue ACE activity.

## Materials and Methods

Adults Wistar rats (10 females and 10 males), body weight 200-250 g were used in the experiment. Animals were housed four or five in cages at room temperature. All animals were with **ad libitum** access to water and a standard laboratory food (14,36 kJ/g; "Sljeme", Croatia) during the entire study. Left-side unilateral nephrectomy was performed by dorsolateral approach under ether anesthesia as described previously (10). Seven days after, the treatment was repeated, the animal was killed by cervical dislocation and right kidney was removed immediately. The left kidney obtained at the time of the initial nephrectomy was used for estimate normal mass (control kidney), and the right kidney for estimate of hypertrophy's mass (hypertrophy kidney). The dry mass of kidney was determined after drying on temperature of 105°C during of 24 hours, and expressed in milligram (absolute mass). The degree of renal hypertrophy is determined by percentage increase absolute mass of kidney (11). The renal capsule removed of every isolated kidney, and its total mass has been measured, and after that it was divided with transversal section into two equal parts approximate. One part of kidney parenchyma in which mass has been measured previously, used to determine dry mass of kidneys. Other part of parenchyma was homogenized and used for determine ACE tissue activity. Blood samples were taken from the tail vein before operation and from abdominal aorta of ether-anesthetized rats on day of sacrifice. After coagulation and centrifugation at 2000 g for 5 min, separately serum was frozen at -25°C and has been used for determine ACE activity and the protein contents. Tissue samples were weighed and washed extensively with 0,9% NaCl solution 4°C) for blood elimination and putted in phosphate buffer, pH 8,3 and stored at 25°C. The tissue samples were homogenized with phosphate buffer in proportion 1: 5 in a Teflon coated Potter-Elvehjem homogenizer by adding 0,05 $cm^3$ a nonionic detergent Nonident P-40 in each sample. Homogenates were centrifuged at 2000 g for 10 min and the supernatants frozen at −25°C until analysis. The ACE activity in serum and tissue was determined by the spectrophotometric method described by Filipović et al. (12), using hippuryl-l-histidyl-l-leucine as substrate („Sigma", St. Louis, USA) and a spectrophometer „Perkin Elmer"550 S for optical readings. The enzyme activity of serum was expressed in units which correspond to 1 nmol of hippuric acid released by hydrolysis of hippuryl-l-histidyl-l-leucine per minute in serum milliliter. The activity of tissue was expressed in units which correspond to 1 nmol of hippuric acid released by enzymatic hydrolysis of hippuryl-l-histidyl-l-leucine per minute in tissue milligram or in protein milligram. The protein concentration was determined by the method of Lowry et al. (13). The results are expressed as the mean values ± SEM. Differences between the means values were statistically compared by Student's t-test, and differences at $p<0,05$ were considered significant. Correlation coefficients were determined by employing Spearman's test. This investigation was done respecting ethical standards.

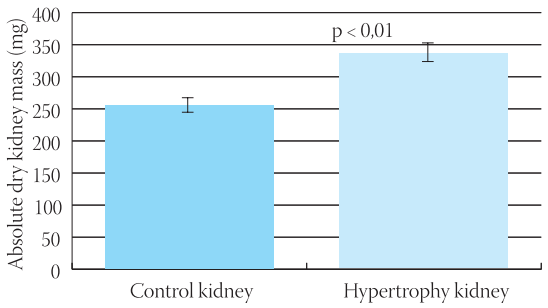


FIGURE 1. Absolute dry mass of kidney (mean ± SEM) before and after compensatory renal growth
Control kidney (n = 20) - nephrectomic kidneys
Hypertrophy kidney (n = 20) - kidneys after 7 days of compensatory renal growth
p = probability

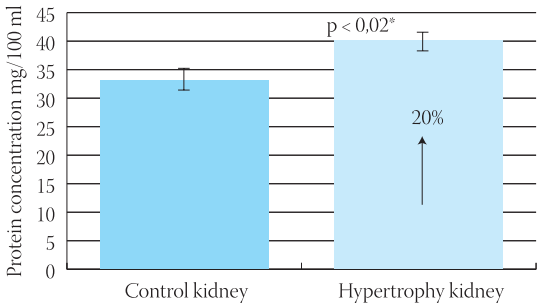


FIGURE 2. The protein contents in renal tissue (mean ± SEM) before and after compensatory renal growth
Control kidney (n = 20) - nephrectomic kidneys
Hypertrophy kidney (n = 20) – kidneys after 7 days of compensatory renal growth
p = probability

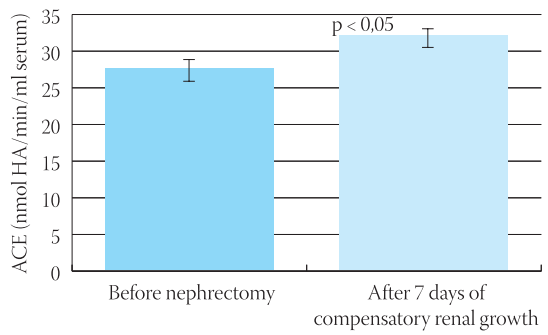


FIGURE 3. Serum ACE activity (mean ± SEM) before and after compensatory renal growth; Before nephrectomy (N=20) – samples of serum before unilateral nephrectomy
After 7 days of compensatory renal growth (N=20) – samples of serum 7 days after unilateral nephrectomy
p = probability

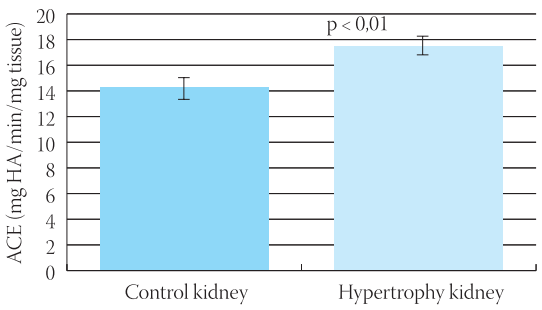


FIGURE 4. Tissue ACE activity (mean ± SEM) before and after compensatory renal growth
Control kidney (n = 20)- nephrectomic kidneys
Hypertrophy kidney (n = 20)- kidneys after 7days of compensatory renal growth
p = probability

## Results

Figure 1. Shows that absolute dry mass of kidney was significantly increased in rats 7 days after unilateral nephrectomy (hypertrophy kidney), and the mean value was 29,9% higher than the values which were determinate in control kidney (p<0,01). As shown in Figure 2. total concentration of protein in kidney's tissue 7 days after unilateral nephrectomy was for 20% higher then before compensatory renal hypertrophy (p<0,02). As shown in Figure 3. ACE activity in serum expressed in units that corresponds to 1 nmol of hippuric acid released by enzymatic hydrolysis of Hip-His-Leu substrate per min/serum milliliter it was significantly higher 7 days after unilateral nephrectomy than before compensatory renal growth, which was statistically significant (p<0,05). To express the ACE tissue activity that corresponds to 1 nmol of hippuric acid released by enzymatic hydrolysis of Hip-His-Leu substrate per min/protein milligram we did not find significantly increased ACE tissue activity 7 days after unilateral nephrectomy (8,7%) in comparison to values that were determinate in control kidney. The ACE activity has been expressed in units that correspond to 1 nmol of hippuric acid released by enzymatic hydrolysis of Hip-His-Leu substrate per min/tissue milligram after 7 days of compensatory renal hypertrophy it was for 23% higher in comparison to values that were determinate in control kidney. This increase of enzymatic's activity was statistically significant (p<0,01). Results have been presented in Figure 4. We found no correlation between the serum and tissue ACE activity in period of compensatory renal hypertrophy.

## Discussion

Compensatory renal growth is a process of adaptation of the remaining kidney to the increased functional demand following unilateral nephrectomy. Results of

our study demonstrated that the absolute dry mass of kidneys (30%) was significantly increased in rats 7 days after unilateral nephrectomy in comparison with values which were determinate in control kidney. Similar results were obtained in other studies (14). The increase of kidney weight, glomerular filtration rate and tubular reabsorption processes are accompanied by an increased cellular metabolic processes catalyzed by several enzymes. Our study showed that serum ACE activity increased for 13% in comparison to value before unilateral nephrectomy. This increase of serum activity was statistically significant. Results of these investigations do not in agreement with the finds Michel et al. (9). These authors showed that ACE activity in serum, which they have been expressed to 1 nmol of hippuric acid released by hydrolysis Hip-His-Leu substrate per min/ mg protein does not change with rats which have been in standard protein diet after unilateral nephrectomy. These authors did not show total concentration proteins in serum in which activity enzyme has been determined. We consider, that the ACE serum activity is expressed in units which are corresponds to 1 nmol of hippuric acid released by enzymatic hydrolysis of Hip-His-Leu substrate per minute/ml serum better criterion for the estimation total enzymatic activity in conditions when concentration of proteins in serum is changing. It is known, that ACE is present on endothelium of many blood vessels, and it is connected with loose electronic forces and that it performs main source serum's ACE. By opposition to the endothelial cells of the human renal vasculature, were no endothelial ACE was detectable, rat kidney vessels showed a strong ACE immunoreactivity in endothelial cells of renal arteries and preglomerular arterial vessels, including the vas afferens (15). The mechanism of the increase serum ACE activity after unilateral nephrectomy is not clear. The increase in blood flow through remnant kidney may be accompanied with a higher enzyme release from the endothelium. Hemodynamic changes of completely cardiovascular system may occur after unilateral nephrectomy and may also play a role in the elevation of serum ACE levels in compensatory renal hypertrophy. In this study, we found that the ACE activity of tissue expressed per milligram of proteins in hypertrophy kidney, increase for only 8% in comparison on value determined in control kidney. However, this increase enzymatic activity does not statistically significant. Results ours investigations have been shown that concentration of proteins in tissue of hypertrophy kidney after 7 days of compensatory renal growth significantly increase in comparison to control kidney. This results are in agreement with explores which were declared early (16). The percentage increment in the protein contents of the hypertrophied kidney increased in proportion to the increase in kidney weight and the protein per gram weight of kidney did not change. Based on these results we can conclude that total tissue enzymatic ACE activity increase in hypertrophy kidney, but the increase in activity of the enzyme is more or less in proportion to the increase in the protein contents of the kidney. The explorations $Na^+$- $K^+$-ATP-ase in basolateral membranes of proximal tubules of kidneys during compensatory renal growth gave similar results. Namely, it noticed that after unilateral nephrectomy increased number transportable units $Na^+$- $K^+$-ATP-ase and that this increase in proportion to the increase in protein contents in cell (17). The expression of ACE activity in units which are corresponding to 1 nmol of hippuric acid released by enzymatic hydrolysis of Hip-His-Leu substrate per minute in milligram of tissue, we established increase enzymatic activity for 23% in hypertrophy kidney in comparison to control kidney which was statistically significant ($p<0,01$). Our results we were not able to compare with other authors because there have not information, in the literature at hand, about tissue ACE activity when it is expressed in units which are corresponded to 1 nmol of hippuric acid released by enzymatic hydrolysis of Hip-His-Leu substrate per minute/ milligram of tissue. Consideration that physiology role of renin angiotensin system in kidney has not sufficient explained, it is very difficultly that to explain causes and mechanisms increase activity this enzyme in compensatory renal hypertrophy on the basis our results. Also, there is question, has increase of tissue ACE activity important role in development adaptive mechanisms in compensatory renal growth or is his increase result development these mechanisms only. Ingelfinger et al. (18) prefer that changes in intrarenal renin angiotensin system can have very important role to keep some homeostasis as in pathogenesis different ill conditions. Michel et al. (9) which were established increase ACE activity in nephrectomic rats on the 16% protein diet in the renal cortex and brush border, suggest that the increase ACE activity in the tubular brush border is specific response to renal ablation. These authors, in yours second experiment, were found significantly increase ACE activity in same tissues in rats on high protein diet. They explain increase activity of this enzyme in brush border in parallel with protein intake. These authors consider that increase ACE activity in brush border accompanying the increased protein intake represents an adjustment to protein hydrolysis within the proximal tubule. Ingelfinger et al. (19) proved high level ACE activity in kid-

ney in experimentally induce nephrosis. It can suppose that increase protein synthesis in hypertrophy cell was associated with increase in ACE synthesis, and that on this way increase its activity in tubules of kidneys. Wolf et al. (4) showed that angiotensin II induces cellular enlargement and stimulates protein synthesis, leading to an increase in total protein contents in culture proximal tubular cells. All these are accepted in the contribution to hypothesis that intracellular angiotensin II as a renal growth factor increase probably to stimulate synthesis ACE and on this way increase its activity. With consideration that angiotensin II has important role in the keeping volume and the structure of body's liquids, to increase ACE activity, concentration of angiotensin II to increase, with aim, to satisfy increase needs reabsorption in tubules of remnant kidney. It is up to examine mechanism of activity renal ACE in normal and compensatory renal growth. Probability there is other mechanisms which are responsible for increase ACE tissue activity, except increase of synthesis. Reduction enzyme hydrolysis, activity of present forms of ACE enzyme probability may also be involved in an increase ACE tissue activity after unilateral nephrectomy. Tufro-McReddie et al. (20) have been posted hypothesis that the renal renin angiotensin system contributes to renal growth during development. On the other hand Wight et al. (21) clear showed that application ACE inhibitor enalapril inhibit compensatory renal growth in adult rats.

## Conclusion

We can conclude that serum and tissue ACE activity increase seven days after unilateral nephectomy. The results our study indicate that ACE, probably has important role in development of adaptive compensatory mechanism after unilateral nephrectomy. Further research will certainly give more information about the role of ACE in compensatory renal growth and mechanisms which are responsible increase ACE activity.

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