EFFECT OF CHLORIDE AND DIAMIDE ON SHEEP KIDNEY, LUNG AND SERUM ANGIOTENSIN CONVERTING ENZYME

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ABSTRACT

The effect of chloride and diamide on angiotensin converting enzyme (ACE) activity from sheep kidney, lung and serum was investigated by using Hip-His-Leu as substrate. Optimum chloride concentrations were 400 - 1000 mM for kidney, 700 mM for lung and 1000 mM for serum. Optimum chloride concentration increased ACE activity of serum and lung 1.70 folds and 2.73 folds respectively of the activity at physiological chloride concentrations, suggesting that the effect of salt on blood pressure may be due to the chloride sensitivity of ACE. The difference in effect of chloride on lung, kidney and serum ACE suggest that each tissue ACE has unique three dimensional structure. Increased pulmonary and serum ACE activity on pretreatment with diamide indicates that tissue oxidation may alter blood pressure.

KEY WORDS

ACE, Serum Chloride, Diamide, Lung.

INTRODUCTION

Angiotensin converting enzyme (EC 3.4.15.1,ACE) catalyzes the hydrolysis of angiotensin I to angiotensin II. ACE and angiotensin II are biologically active components of renin - angiotensin –aldosterone system (RAS) which play central role in the maintenance of blood pressure and electrolyte and water homeostasis(1). Distribution of ACE varies among tissues in any given mammalian species for unknown reasons. For example, kidney has almost five fold more ACE activity than lung in man (2) and sheep(3). One of characteristic property of ACE is its requirement for monovalent ions particularly chloride for catalytic activity(4). The mechanism by which chloride activates ACE has not been established clearly(5). Further, effect of chloride on serum ACE from eight mammalian species with varried ACE activity showed differences in optimum chloride concentration among species(6). Relationship between difference in ACE activity among tissues and chloride dependence is unknown in any animal species. Salt restriction plays critical role in controlling

hypertension and improves effectiveness of anti hypertensive drugs in some cases(7). Since the chloride is an integral part of salt the effect of salt on blood pressure may be due to the chloride dependency of ACE. However the mechanism by which salt influences blood pressure is unknown. Therefore the influence of chloride on sheep ACE from lung, kidney and serum was studied. Further, lung ACE is major contributor of circulating angiotensin II in mammals(1,2). Though the lungs are directly exposed to oxygen , effect of O₂ or oxidation - reduction system on lung ACE activity is unknown. It was reported that, ACE activity is affected by oxidation or oxygen which removes endogenous substances or inhibitors that exist in tissues(8). Sulfhydryl moiety is essential to these substances to exert inhibitory activity on ACE(9). Since diamide is a sulfhydryl oxidizing agent, the possible presence of these inhibitors in sheep kidney, lung and serum was analyzed with diamide.

MATERIALS AND METHODS

Hippuryl - L - Histidyl - L - Leucine (HHL), Hippuric acid (HA), diamide and bovine serum albumin were obtained from Sigma Chemical Company, U.S.A. Other reagents were analytical grade commercial chemicals. Tissues were obtained from local slaughter house and kept deep frozen at – 70°C. Only parts of the tissues free from any disease process were used in the study. Serum was separated by centrifugation within

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1 hour. Deionized water was used.

ACE was extracted from tissues as described earlier(3). The extraction buffer has the following composition : 0.1 M phosphate buffer pH 8.3, 50mM NaCl. Protein content of the tissue extract was measured by the method of Lowry et al (10) using bovine serum albumin as standard.

Tissue ACE activity was measured with HHL as substrate by a method modified from Cushman and Cheung(11). The reaction mixture (0.175 ml) contained 0.1ml of 5mM HHL dissolved in 0.2M phosphate buffer pH 8.3 containing 0.6M NaCl, tissue extract and deionized water. The tissue extract in volume of 25 µl or less was used to initiate the reaction. After 30 minutes incubation at 37°C the reaction was arrested by adding 0.175 ml of 1M HCl. Hippuric acid was extracted and resuspended in ethanol and measured spectrophotometrically at 228nm as described earlier(3).

One unit of ACE was defined as the amount of enzyme catalyzing the release of 1n mole of hippuric acid from HHL per minute at 37°C. The specific sample volume, 20 µl for kidney (Protein content 90-110µg), 25 µl for lung (Protein content 180-210 µg) and 25 µl for serum (Protein content 1.75-2mg) was chosen by executing curves at increasing sample volume.

To study the effect of chloride on the ACE activity, tissue extracts were prepared in chloride free buffer and dialysed against same buffer along with serum for over night at 4°C. The chloride free extracts were assayed for ACE activity in the absence of chloride at physiological chloride concentration and at different chloride concentrations.

The final chloride concentrations used to study the effect of chloride on pulmonary ACE, renal ACE and serum ACE activity and concentrations of NaCl solutions used are shown in

Table 1 : Concentrations of NaCl solutions (mM) used to study the effect of chloride on sheep kidney, lung and serum ACE activity

Final Chloride (mM)	6	10	100	500	1000	1500
Required NaCl (mM)	21	35	350	1750	3500	5250

Table 1. 50 µl of NaCl prepared in 0.2M phosphate buffer pH 8.3 was added to each tube. To calculate concentrations of NaCl solutions the chloride of dialysed tissue extract and phosphate buffer was not considered (Chloride concentration < 2meq /l). The concentration of chloride in the tissue extracts, serum and buffer was determined with Technicon chloride auto analyzer model RA-2000.

To study the effect of diamide on ACE activity tissue extracts and serum were pretreated with diamide. 10 µl of 100 mM diamide (Prepared in 0.1M phosphate buffer pH 8.3) were added to 90 µl of tissue extract or serum to give 10mM final-concentration(6). 10µl of deionized water were added to blank tubes. Tissue extracts and serum were chilled for 30 minutes at 0°C and used for determination of ACE activity.

RESULTS

Hippuric acid production was linear with increasing volume of tissue extract or serum (enzyme) and it was also linear for 30 minutes incubation time (data are not shown). Further freezing and thawing had no effect on ACE activity. The curves of chloride concentration *versus* ACE activity of lung, kidney and serum are shown in Fig 1. The curves of lung, Kidney and serum ACE activity at optimum chloride-concentrations are different. The ACE activity of kidney increased rapidly to reach maximum and then it decreased slowly. In contrast, the serum ACE activity increased gradually to reach maximum and then it decreased. Further, the curve of kidney ACE presented plateau in the maximum point. The curve of lung ACE had a special form, it presented increase and decrease of ACE

Table 2 : Effect of chloride on sheep kidney, lung and serum ACE activity

Tissue	Optimum (Cl) mM	ACE activity* at Optimum (Cl) mM	ACE activity* in the absence of (Cl) mM	ACE activity* at Physiological (Cl) mM	Physiological (Cl) mM	ACE activity at Optimum (Cl) mM ACE activity at Physiological (Cl) mM	ACE activity at Optimum (Cl) mM ACE activity in the absence of (Cl) mM
Kidney	400 - 1000	24.00 ± 2.50	3.77 ± 0.45	12.80 ± 1.50	10.0 ± 1.00	1.88 ± 0.35	6.40 ± 1.00
Lung	700	17.48 ± 1.20	2.85 ± 0.10	6.40 ± 0.75	6.0 ± 1.50	2.73 ± 0.40	6.13 ± 0.75
Serum	1000	4.85 ± 0.50	0.42 ± 0.05	2.85 ± 0.15	100 ± 10.00	1.70 ± 0.30	11.54 ± 2.50

* ACE activity is expressed as n mol / ml / min. Data are expressed as Mean ± SD; n = 6

activity with increasing chloride concentration. However all three curves presented only one maximum point of activity. The optimum chloride concentrations obtained were 700mM for lung ACE and 1000mM for serum ACE respectively. For the kidney ACE optimum chloride concentration was in the range of 400-1000mM (Table 2, first column). Maximum ACE activity (nmolHA/ml/min) under optimal chloride concentration was 24 for kidney, 17.48 for lung and 4.85 for serum (Table 2, second column). In the fourth column of the Table 2, ACE activity of kidney, lung and serum obtained at physiological chloride concentrations are shown. ACE activity of kidney, lung and serum obtained in the absence of chloride are shown in the third column of Table 2. In the absence of chloride ACE activities of kidney, lung and serum were 29.40, 44.45 and 14.76% respectively of the activities observed at physiological chloride concentration. Under optimum chloride concentration ACE activity of kidney, lung and serum increased approximately two folds (Table2, sixth column). However, the presence of chloride at optimum concentration increased ACE activity of kidney, lung 6.0 folds and serum 11.54 folds respectively of the activity observed in the absence of chloride (Table 2, seventh column). These results indicate that even though activities of kidney, lung and serum ACE were increased in the presence of chloride, the serum ACE required high chloride concentration and exhibits maximum increase in activity whereas kidney and lung ACE required low concentration and shows minimum increase in activity.

The effect of diamide on ACE activity of lung, kidney and serum is shown in Table 3. Pretreatment with diamide enhanced ACE activity of serum and lung only. However, the increase in ACE activity of the serum on pretreatment with diamide was two times more than that of lung ACE. Since diamide is sulfhydryl oxidizing agent these results suggest that ACE activity of serum is more susceptible to inhibition by sulfhydryl containing compounds of endogenous or exogenous origin while kidney ACE is least susceptible.

Table 3 : Effect of diamide on sheep kidney, lung and serum ACE activity (values are mean $\pm$ SE of 6 determinations)

Tissue	ACE activity n mol HA / ml / min		Enhancement of activity (%)
	without diamide	with diamide	
Kidney	20.48 $\pm$ 1.00	21.57 $\pm$ 1.08	5.32
Lung	13.90 $\pm$ 0.33	16.04 $\pm$ 0.25	15.40
Serum	3.43 $\pm$ 0.70	4.72 $\pm$ 0.80	13.61

DISCUSSION

The ACE activity measurements for kidney, lung and serum

were performed in the linear parts of HA production *versus* tissue extract volume and incubation time. Enhancement in lung, kidney and serum ACE activities at physiological chloride concentration suggest non-essential activator function for chloride for each ACE(12). There was no relationship between differences in kidney, lung and serum ACE activity(3) and optimal chloride concentration or tissue chloride concentration. However, the different optimum chloride concentrations and differences in the chloride concentration curves obtained for lung, kidney and serum ACE indicate that the sensitivities of two domains of ACE to chloride in each tissue is different and each domain may consist of unique structure. Further, the high K_m values of serum and lung ACE(14) and their high chloride requirement to exhibit maximum activity suggest that the chloride induces conformational change in ACE molecule allowing more favourable binding of low affinity substrate to active site(13). In contrast, low K_m value of kidney ACE (14) and its low chloride requirement to exhibit maximum activity suggest that substrate with high affinity for ACE do not require much of conformational change in order to bind at active site and hence less dependence on chloride(5). Since, interaction of chloride with cationic binding site of ACE is restricted by the size of anion(4,15) the differences in optimum chloride concentrations for lung, kidney and serum ACE indicates differences in their conformation. Therefore, these differences in effect of chloride on lung, kidney and serum ACE indicate

Figure 1 : Effect of chloride on pulmonary, renal and serum ACE activity. Assays were performed with 5mM HHL prepared in 0.2M phosphate buffer pH 8.3
 Δ - Δ Kidney ACE, $\circ$ - $\circ$ lung ACE, $\bullet$ - $\bullet$ serum ACE.
 Each dot shows mean of six determinations.

that each tissue ACE has unique three dimensional structure.

Since ACE activities of lung, kidney and serum ACE are increased with increase in chloride concentration, high salt intake may raise extracellular chloride level which causes activation of ACE resulting excess angiotensin II formation and high blood pressure. In contrast, salt restriction may lower extracellular chloride level thus leading to less formation of angiotensin II and hypotension. Therefore, the effect of salt on blood pressure could be due to chloride sensitivity of ACE. Since molecular structures of sheep and human tissues ACE are unknown, further studies are needed for confirmation. Increase in serum ACE activity on pretreatment with diamide indicates presence of endogenous inhibitors for serum ACE. Further, increase in pulmonary ACE activity on pretreatment with diamide suggest that tissue oxidation or tissue damage involving oxidation may be a factor affecting ACE activity and blood pressure because pulmonary ACE is the major-contributor of angiotensin II to circulating RAS(12).

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