Familial Hyper-angiotensin Converting Enzyme (ACE)-emia: Increased Production of ACE by Monocyte-macrophage

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We report here a familial clustering of elevated serum angiotensin converting enzyme (ACE) levels. The patient was a 58-year-old Japanese female. She had been in excellent health until the age of 45, when she noticed a decrease in visual acuity of her left eye. Despite intensive therapy under the diagnosis of occlusion of the central retinal vein, she lost her visual acuity at the age of 45. Thereafter, she has been in excellent health. The only abnormality found in this case has been a markedly elevated level of serum ACE (625 n mol/min/ml; normal range; 22–40 n mol/min/ml of serum). Her blood pressure was within normal limits (140/80 mmHg). There was no evidence for the diagnosis of sarcoidosis, Gaucher's disease, leprosy, hyperthyroidism, diabetic retinopathy, or liver disease. One of her two sisters also showed a marked increase in serum ACE activity (303 n mol/min/ml), and remarkably high levels of serum ACE (276 and 294 n mol/min/ml) were demonstrated in both of two sons of this sister. All the members of this family have been in excellent health. The serum ACE activity was activated by chloride and cobalt ions, and inhibited by EDTA, captopril and rabbit antiserum to purified human plasma ACE. Thus, our study showed a familial clustering of "hyper-ACE-emia", and the disorder appears to have been inherited as an autosomal dominant trait.

Key Words: Angiotensin II

Angiotensin converting enzyme (ACE), a dipeptidyl carboxypeptidase has been demonstrated in vascular endothelial cells¹⁾, proximal renal tubules²⁾, and macrophages³⁾. The enzyme converts angiotensin I to angiotensin II and inactivates bradykinin. The activity was initially discovered to increase in the serum of patients with sarcoidosis^{4,5)}. Subsequently, serum ACE activity was found to be elevated in patients with a variety of diseases, including Gaucher's disease⁶⁾, leprosy⁷⁾, silicosis⁸⁾, hyperthyroidism⁹⁾, diabetic retinopathy¹⁰⁾, and liver diseases¹¹⁾.

We describe in this communication an individual with a markedly elevated serum ACE level and three family members who have similarly elevated levels of serum enzyme. Although the patient had a central retinal vein occlusion, neither she nor her family members had a disease that is conven-

tionally associated with elevated levels of serum ACE.

MATERIALS AND METHODS

Serum:

Blood was collected from antecubital veins and serum was separated immediately after blood-collection and stored at -20°C for ACE measurements. Venipuncture in patients and normal subjects was performed after informed consent for the procedure was obtained.

ACE assay:

Conditions were previously described¹²⁾ and were based on a spectrophotometric assay and the substrate hippuryl-L-histidyl-L-leucine¹³⁾. ACE activity was determined as follows: to a 10×75 mm glass test tube was added 0.15 ml of buffered substrate containing 0.165 M borate-borax, pH 8.3,

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0.5 M NaCl, and 8.33 mM hippuryl-L-histidyl-L-leucine. The solution was warmed to 37°C in a water bath and the reaction was initiated by addition of 0.1 ml of samples. The reaction mixture was stirred in a vortex mixer for one second and incubated at 37°C for 30 min. Reaction was terminated by addition of 1.5 ml HCl (1 N) and cooling in a ice-bath. The product hippuric acid was extracted with 1.5 ml ethylacetate and quantitated by a spectrophotometer. One unit was defined as one nanomole hippuric acid product per min at 37°C under the standard assay conditions.

Hippuryl-L-histidyl-L-leucine was purchased from Protein Research Foundation, Osaka, Japan. Other reagents were analytical grade obtained from commercial sources.

Characterization of the enzyme:

To assess the effect of Cl^- on the activity of the enzyme, samples were dialyzed against Cl^- -free potassium phosphate buffer (1 mM, pH 8.3), and the incubation mixtures were made up with a range of NaCl concentrations from 0 to 800 mM. The effect of the inhibitors, EDTA and SQ 14225 (E.R. Squibb & Sons, New York) was assessed on the enzyme activity. To see the effect of divalent metal ions, the samples were incubated with various concentrations of CoCl_2 for 30 min at 37°C prior to assay, which was initiated by addition of hippuryl-L-histidyl-L-leucine.

Effect of anti-ACE antibody on the enzyme activity:

Antiserum against human plasma ACE was prepared in rabbits as previously described^{14, 15}. Monospecificity was established by Ouchterlony double diffusion and confirmed by enzyme linked immunosorbent assay¹⁶. The samples were incubated with anti-ACE for 30 min at 37°C in 200 μl of 0.375 M NaCl-0.125 potassium phosphate buffer, pH 8.3, and assayed for residual activity. The assayed activity was corrected for residual activity in the rabbit antiserum.

Estimation of molecular weight of the enzyme:

We determined the approximate molecular weight of serum ACE using a column packed with Sephacryl S-300 (1.5 cm $\times$ 86 cm column). Liquid chromatography was run at 4°C. We transferred 4 ml of serum (625 units/ml) to a Sephacryl S-200 column (0.9 $\times$ 96 cm; flow rate = 6 ml/hr) equili-

brated with 0.05 M phosphate buffer (pH 7.0), containing 0.5 M NaCl. The active fractions were pooled, dialyzed against 0.01 M phosphate buffer (pH 8.0), and applied to a column of DE 52-cellulose (1.5 $\times$ 43.5 cm) which was equilibrated with the same buffer. The enzyme was eluted with a linear gradient of KCl from 0 to 300 mM in the same buffer. The active fractions were pooled, concentrated with an Amicon YM-10 filter and applied to the Sephacryl S-200 column equilibrated with 0.015 M phosphate buffer, pH 7.0, containing 0.5 M NaCl. The active fractions obtained by the re-chromatography on Sephacryl S-200 were concentrated (500 n mol/min/mg protein), and applied to a Sephacryl S-300 column (1.5 $\times$ 86 cm) equilibrated with the same buffer (flow rate = 6 ml/hr) to estimate the approximate molecular weight.

RESULT

Case history:

The patient was a 58-year-old Japanese female who had been in excellent health until the age of 45, when she noticed a decrease in visual acuity of her left eye. A diagnosis of occlusion of the left central retinal vein was made, and this disease was treated ineffectively with corticosteroid therapy. Occlusion of the central retinal vein later occurred in her right eye, and she lost visual acuity of this eye due to hemorrhage into the optic fundus. Except for the ocular changes, routine physical examination was within normal limits. Her blood pressure was 140/80 mmHg. There was no lymphadenopathy, splenomegaly or hepatomegaly. A chest X-ray was within normal limits. Biopsy specimens of the scalene lymph nodes, skin and muscle were within normal limits. With the exception of decreased visual acuity, she has been in excellent health, thereafter.

Laboratory findings:

Routine laboratory studies, including white cell count, hematocrit, hemoglobin, platelet count, urinalysis, serum protein, BUN, creatinine, serum electrolytes and liver function studies, were within normal limits (Table 1). The only abnormality found in the patient was an extremely high level of serum ACE (625 n mol/min/ml) (Table 2). The high ACE level has been sustained for nine years of

Table 1. Routine laboratory examinations

White-cell count 4,500 (neutrophils 60%, banded forms 3%, lymphocytes 27%, monocytes 8%, eosinophils 2%).

Hematocrit 39.7%, Hemoglobin 13.5 g/100 ml, Platelet-count 300,000.

Urinalysis, normal.

Blood chemistry: Total protein 7.6 g/100 ml, Albumin 4.6 g/100 ml, Urea nitrogen, 13.0 mg/100 ml, Creatinine 0.9 mg/100 ml, Uric acid 4.1 mg/100 ml, Sodium 145 m mol, Potassium 4.3 m mol, Chloride 103 m mol, Calcium 9.6 mg/100 ml, Inorganic phosphorus 3.9 mg/100 ml, Glutamic Oxaloacetic Transaminase (GOT) 22 U/l, Glutamic Pyruvic Transaminase (GPT) 14 U/l, Lactate Dehydrogenase (LDH) 197 U/l, Alkaline Phosphatase 66 U/l, Acid Phosphatase 3.4 K-A Unit. Serum Iron 74 μ g/100 ml.

Table 2. Other laboratory examinations

Plasma renin activity	1.1 ng/ml/hr (normal range = 0.5–2.0)
Angiotensin I	120 pg/ml (normal range = less than 200).
Angiotensin II	96 pg/ml (normal range = less than 110).
Angiotensin converting enzyme	625 n mol/min/ml (normal range = 20–40).
Aldosterone	98 pg/ml (normal range = 30–160).
Thyroid hormones	
T ₃	8.7 μ g/100 ml (normal range = 4.5–12).
T ₄	125 ng/100 ml (normal range = 80–180).

follow-up. Such a high level of serum ACE has not been found in any other disease examined in our laboratory, including sarcoidosis, leprosy, liver diseases, or hyperthyroidism (Table 3). To see whether such a high ACE level in the blood may alter components of the renin-angiotensin system, angiotensin I, angiotensin II, and aldosterone were determined by specific radioimmunoassays. Angiotensin I, angiotensin II, or aldosterone was not increased in the patient serum (Table 2). Plasma renin activity was also within normal limits (Table 2). Thyroid hormone levels in the serum were within normal limits. Acid phosphatase was not increased in the serum (Table 1). These observations strongly suggested that she was free of diseases such as sarcoidosis, hyperthyroidism, leprosy,

Table 3. Serum angiotensin converting enzyme (ACE) levels in various diseases

Sarcoidosis	Active	53 $\pm$ 14 n mol/min/ml (n=64) (mean $\pm$ S.D.)
	Inactive	29 $\pm$ 7 n mol/min/ml (n=21)
Hyperthyroidism		65 $\pm$ 18 n mol/min/ml (n=21)
Silicosis		45 $\pm$ 10 n mol/min/ml (n=29)
Liver cirrhosis		48 $\pm$ 11 n mol/min/ml (n=24)
Leprosy		31 $\pm$ 13 n mol/min/ml (n=50)
Tuberculosis		37 $\pm$ 9 n mol/min/ml (n=25)
Normals		31 $\pm$ 9 n mol/min/ml (n=63)

Gaucher's disease, or liver disease.

Characterization of the serum ACE:

To examine whether the high ACE activity in the serum is a true ACE, partial purification and characterization of the serum ACE activity was carried out. The enzymatic activity in the serum closely resemble classical ACE in several respects. It was inhibited by EDTA (Fig. 1) and by captopril (Fig. 2). 50% inhibition by captopril occurred at at 6.2 nM. It was activated by chloride with a half maximal effect at less than 200 mM (Fig. 3). It was also activated more than 200% by cobalt ions (Fig. 4). The activity was inhibited 80% by a

Fig. 1. Inhibition of ACE by EDTA (●—● serum from the patient, ×—× serum from a normal subject).

Fig. 2. Inhibition of ACE by captopril (SQ 14,225) (●—● serum from the patient, ×—× serum from a normal subject).

Fig. 3. Cl^- activation of ACE derived from patient's serum.

Fig. 4. Co^{2+} activation of ACE derived from patient's serum.

Fig. 5. Inhibition of ACE by rabbit antiserum to human plasma ACE. ●—● serum from the patient, ○—○ partially purified ACE from patient's serum, □—□ serum from a normal subject.

rabbit antiserum prepared against purified human plasma ACE (Fig. 5).

To estimate the molecular weight of serum ACE from the patient, the partial purification was carried out as described in Materials and Methods. Partially purified ACE (500 n mol/min/mg protein) was applied to a Sephacryl S-300 column. The molecular weight was estimated at about 300,000, by using ferritin, catalase, and bovine serum albumin as protein markers (Fig. 6).

Family study:

Because we could not find any abnormality which may cause an increase in serum ACE in this case, her family members (6 of 7 persons) were subjected to study. Interestingly, one of her two sisters showed a marked increase in ACE activity in the serum (303 n mol/min/ml). Remarkably elevated ACE levels (276 and 294 n mol/min/ml) were also found in both of two sons of this sister. Serum ACE levels were within normal limits in the other sister (39.2 n mol/min/ml) and in one of her

Fig. 6. Gel-filtration profile of partially purified ACE on a Sephacryl S-300 column. Ferritin, catalase, and bovine serum albumin were used to estimate the approximate molecular weight.

two sons (36.9 n mol/min/ml). The other son of this sister showed a slight increase in serum ACE activity (58.9 n mol/min/ml). None of the members of this family showed evidences suggestive of sarcoidosis, Gaucher's disease, hyperthyroidism, silicosis, leprosy, or liver disease. They had no eye disease. There was no history of consanguinity.

DISCUSSION

This study showed a familial clustering of high ACE levels in the serum. In four of seven family

members, serum ACE levels were extremely elevated (374 ± 72.4 n mol/min/ml), in comparison with those of patients with other diseases such as sarcoidosis (Table 3). The propositus had suffered from occlusion of the central retinal vein at the age of 45, but she has been in excellent health, thereafter, while the extremely high ACE level in the serum has been sustained. The other family members have also been in excellent health. The only abnormality found in the family members was also a remarkable increase in serum ACE activity. Despite the extremely high level of serum ACE, blood pressure was within normal limits. Plasma renin activity, angiotensin I, angiotensin II, and aldosterone in the blood were within normal limits. These findings suggested that circulating ACE did not play an important role in blood pressure regulation¹⁷. That the increased enzyme measured in this study is a true ACE is established by several criteria, including chloride dependency, activation by cobalt ions, and inhibition by EDTA, captopril (a specific ACE inhibitor) and monospecific antiserum to human plasma ACE^{13, 18, 19}.

The major baseline source of ACE in serum is believed to be the vascular endothelial cells, which are known to contain large amounts of ACE activity²⁰. Recently, it has been shown that serum ACE activity increases after administration of bleomycin. Electron microscopic studies showed that swelling and death of endothelial cells occurred between days 2 and 5 following an intravenous dose of bleomycin^{21, 22}. Injury to the vascular endothelial cells may cause an increase in serum ACE activity. Recently, in some cases of diabetic retinopathy, serum ACE levels have been shown to be elevated¹⁰. Generalized extensive vasculization may occur and may play a role in the elevation of serum ACE levels in patients with diabetic retinopathy. In the present study, the propositus or the family members did not show any evidence suggestive of diabetes. Only vascular disease found in this study, was occlusion of the central retinal vein found in the propositus, when she was 45-year-old. Thereafter, she has been, however, in excellent health, despite the long-standing elevation of serum ACE. With the exception of the propositus, there was no history of vascular disease in the family members. Causal relationship between occlu-

sion of the central retinal vein and the high serum ACE level, is therefore, not probable.

It is well known that granulomatous lymph nodes from sarcoidosis²³, and spleen from patients with Gaucher's disease contain large quantities of ACE, which is also generally increased in the patients' serum⁴⁻⁶. The sources of excess serum ACE are believed to be the sarcoid epithelioid cells^{24, 25} and Gaucher's cells²⁶, both mononuclear phagocytes that normally contain negligible ACE^{17, 33}, but are inducible for ACE synthesis in culture^{27, 28}. These observations suggest that monocyte-macrophages may be another source for serum ACE. The propositus or the family members have not shown abnormalities suggestive of sarcoidosis, leprosy, or Gaucher's disease. In liver disease, serum ACE levels have been shown to be elevated¹¹. The mechanism by which serum ACE levels have been elevated have not been elucidated. Liver has not been demonstrated to be a source of ACE production²⁹. The liver may inactivate serum ACE. Impaired hepatic clearance in diseases of the liver may increase serum ACE activity¹¹. In our cases, no abnormal findings have been demonstrated by routine liver function tests.

Serum ACE seems to be under some humoral regulation, since it is elevated in hyperthyroidism⁹. It is also elevated in serum of patients treated with the enzyme inhibitor, captopril, suggesting that ACE is under feedback regulation by a reaction product such as angiotensin II^{30, 31}. In our study, any of the cases has not been affected by hyperthyroidism, or treated with captopril. Little is known about the turnover of ACE in serum even in normal subjects. The level of this enzyme in serum is remarkably stable and is replenished within 5-7 days after the level is lowered by plasmapheresis³². Further studies are required for elucidation of the cause and source of the increased serum ACE in our cases. If elevated ACE in the cases does represent overproduction by certain cells, such as endothelial cells or monocyte-macrophages, it raises an interesting question regarding the mechanism that regulates the production of this protein. Recently, we discovered that peripheral blood monocytes released a significant amount of ACE in patients with sarcoidosis³³. Thus, we examined ACE production by circulating mono-

Fig. 7. Release of ACE by circulating monocytes from the patient. Monocytes were prepared by incubating the mononuclear cell fraction isolated by Ficoll-Hypaque density gradient centrifugation as described (33). Isolated monocytes were incubated at 37°C in a serum-free F-10 medium. Incubation medium was harvested everyday for ACE assay and the cells were refed with fresh F-10 medium.

cytes in this case. Peripheral blood monocytes from the proband released a high ACE activity (Fig. 7). This disorder may be valuable for study of the mechanisms by which ACE levels are elevated in serum. Since the disorder appears to have been inherited as an autosomal dominant trait, it may serve as a useful model for study on the expression of ACE gene(s) in monocytes.

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