Reninlike Enzymes in Human Vasculature

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The present study was designed to identify angiotensin I (Ang I)–forming angiotensinogenases in human extrarenal vasculature and to examine the theory of Jiménez Díaz on their stimulation in essential hypertension. Vascular sections obtained intraoperatively from 14 normotensive and 16 hypertensive patients undergoing corrective surgery, 68 umbilical cord blood vessels from parturient women, tissue samples from nine explanted hearts, and serum from anephric and healthy individuals were investigated. Ang I-forming angiotensinogenase activities were determined enzyme-kinetically by using Ang I radioimmunoassay and purified sheep or human angiotensins. Three nonrenin Ang I-forming angiotensinogenases (pH optima of 4.0, 5.1, and 6.1) were identified in extrarenal vasculature, in cardiac tissues, and in plasma. Highest specific activities of nonrenin Ang I-forming angiotensinogenase (in nanograms Ang I per gram times hour; mean±SD) were found in cardiac tissue (2,821±497, n=9), followed by carotid artery intima (1,448±982, n=10), arteries (1,307±736, n=18), and umbilical cord arteries (135±55, n=35). Extrarenal arterial Ang I-forming angiotensinogenases were linearly correlated with those of local angiotensin converting enzyme and plasma renin activity. In essential hypertension, extrarenal arterial Ang I-forming angiotensinogenases were scattered, but not generally stimulated. The data obtained indicate the existence of nonrenin Ang I-forming angiotensinogenases in human extrarenal vasculature, in kidney, and in plasma. The postulate of stimulation of extrarenal arterial Ang I-forming angiotensinogenases in essential hypertension cannot be supported. Similar to the classification of plasma renin activity, a classification of Ang I-forming angiotensinogenase activity is proposed, consisting of patients with essential hypertension divided into subgroups exhibiting high, normal, or low vascular Ang I-forming angiotensinogenase activities. (Hypertension 1990;15:848–853)

There is increasing evidence that the octapeptide angiotensin II (Ang II), the biologically active component of the renin-angiotensin system, is not exclusively formed in plasma but also in various tissues. Almost half a century ago, it was postulated that an angiotensin I (Ang I)–forming enzyme is present in extrarenal arterial walls and that this participates in the pathogenesis of essential hypertension.1 In the meantime, Ang I-forming angiotensinogenases have been demonstrated in extrarenal vasculature of various animal species.2 Furthermore, it has been found that extrarenal arterial wall Ang I-forming angiotensinogenases, biochemically different from renal renin, are stimulated in spontaneously hypertensive rats.3,4 Recently, we noticed in the rat that those nonrenin Ang I-forming angiotensinogenases identified in extrarenal arteries are also contained in the kidney, although renal renin was scarcely demonstrable in extrarenal vasculature (authors’ unpublished observations).

The intention of the present study was to identify Ang I-forming angiotensinogenases in human extrarenal vasculature, to establish whether these enzymes are also present in kidney, heart, and plasma, and to determine whether they are affected in essential hypertension.

Methods

Human vascular sections were obtained from individuals undergoing corrective surgery. Patients with essential hypertension were compared with normotensive patients; patients with secondary forms of hypertension were excluded. Routine biochemistry was normal in this population (including lipid values). In all hypertensive patients, antihypertensive therapy was discontinued for at least 1 week before surgery.
Umbilical cord blood vessels derived from partrial women and cardiac tissues from explanted hearts that were not suitable for transplantation were used in this study. Plasma was collected from anephric patients and healthy individuals.

The tissues were cut into small pieces approximately 0.5 mm thick; they were homogenized in 0.9% saline (1:8) with a Potter-Elvehjem homogenizer (Braun, Melsungen, FRG) (six times at 30 seconds each) and subsequently with an ultrasonic disintegrator (MSE Ltd., Crawley, Sussex, England) (six times at 1 second each, 6 μm). The suspensions were centrifuged at 8,050 g for 1 minute, and the supernatants were used. All procedures were carried out below 4°C.

Ang I-forming angiotensinogenase activities were measured enzyme-kinetically by using the Ang I radioimmunoassay and sheep or, in some cases, human angiotensinogen. Renin substrates were prepared as described previously. Tissue extract (0.1 ml) was incubated at 37°C with 0.1 ml of 0.3 M phosphate buffer containing the angiotensinogen (approximately 800–1,200 ng/ml) and angiotensinase inhibitors. Final concentrations were (M) EDTA 3.3, 8-hydroxyquinoline sulfate 10.5, 2,3-dimercaptopropanol-1 4.9, and diisopropyl fluorophosphate 3.6. Plasma renin activity was likewise determined but without the addition of angiotensinogen. Angiotensin converting enzyme was assayed by the fluorimetric method of Piquilloud et al with Z-Phe-His-Leu as substrate. Ang I-degrading enzyme activities were quantified as described recently. Separations and biochemical characterizations of the enzymes were achieved by fast protein liquid chromatography (equipment, including columns and Polybuffer, was obtained from Pharmacia, Uppsala, Sweden) with the anion exchange column (Mono Q HR 5/5; buffer system: 0.02 M Tris/0.02 M Tris+1 M NaCl, pH 7.4), the chromatofocusing column (Mono P HR 5/20; start buffer: 0.025 M Bis-Tris, pH 7.2; elution buffer: Polybuffer 74, pH 4.0), and the gel filtration column (Superose 6 HR 10/30; elution buffer: 0.05 M phosphate+0.15 M NaCl, pH 7.2).

Statistical analysis and significance of results were calculated by standard methods. Student’s t-test was used for paired or unpaired samples, as appropriate, and for correlation coefficients.

Results

Some enzyme kinetic assays of tissue Ang I-forming angiotensinogenases are illustrated in Figure 1 (left panel). The slopes of the resulting straight lines represent the enzymatic activities. The incubations were performed at the respective optimal pH values in the presence of sheep or human angiotensinogen. The effects of pH on Ang I-forming angiotensinogenase activities in various tissue extracts and in plasma are demonstrated in Figure 1 (right panel). The measurements shown here were performed exclusively with human angiotensinogen. A total of four pH optima were determined: at pH 4.8 (4.0), pH 5.4 (5.1), pH 6.2 (6.7), and an additional one for cardiac tissue at pH 6.1 (the values obtained with sheep angiotensinogen are expressed in parentheses). To depict the results of various studies within one diagram and to demonstrate the
Figure 2. Graph showing fast protein liquid chromatography of angiotensin I (AI)-forming angiotensinogenases in human kidney cortex extract. Starting material was 28 mg protein; anion exchange column was Mono Q HR 5/5; buffer system contained 0.02 M Tris/0.02 M Tris+1 M NaCl, pH 7.4. Sample (0.1 ml) was incubated at 37°C with 0.1 ml of 0.3 M phosphate buffer containing angiotensinase inhibitors and sheep renin substrate (approximately 400 ng/ml) at pH 4.0, 5.1, and 6.75 (three assays).

Figure 3. Scatterplot showing distribution of specific human vascular and cardiac angiotensin I (AI)-forming angiotensinogenase activities. Tissue extract (0.1-ml sample (0.1 g tissue wet wt/0.8 ml of 0.9% NaCl) was incubated at 37°C with 0.1 ml of 0.3 M phosphate buffer containing angiotensinase inhibitors and sheep renin substrate concentration (approximately 1,200 ng/ml). Incubation pH was 5.1 for carotid artery intima and for extrarenal artery and vein, 5.4 for umbilical cord vessels, 6.1 for heart, and 6.7 for serum.

Figure 5 compares extrarenal arterial Ang I-forming angiotensinogenase activities of patients with essential hypertension with normotensive controls. Individual values and their arithmetical means are depicted separately for both enzymes (pH optima, 5.1 and 4.0). No significant differences were obtained, although activities tended to decline in carotid artery intima of hypertensive patients. To test whether relations exist between both vascular enzymes or between them and plasma renin or other components of the renin-angiotensin system, the respective correlation coefficients \( r \) and probabilities \( p \) were evaluated. The results are as follows: arterial Ang I-forming angiotensinogenase (pH optimum 5.1) compared with arterial Ang I-forming angiotensinogenase (4.0): \( r=0.73, p<0.001, n=35 \); arterial Ang I-forming angiotensinogenase (5.1) compared with plasma renin activity: \( r=0.8, p<0.05, n=6 \); arterial Ang I-forming angiotensinogenase (5.1) compared with plasma renin activity: \( r=0.97, p<0.001, n=6 \); carotid artery intima Ang I-forming angiotensinogenase converting enzyme: \( r=0.78, p<0.01, n=10 \); carotid artery intima Ang I-forming angiotensinogenase (4.0) compared with carotid artery intima angiotensin converting enzyme: \( r=0.6, p<0.05, n=10 \).

Figure 6 illustrates the effects of pH on human plasma Ang I-forming angiotensinogenase activities. In plasma of healthy individuals (middle panel), two major equal pH optima were determined at 6.2 and 5.3, and a minor one was determined at 7.2. In plasma of bilaterally nephrectomized patients (left panel), pH optima were not found at 6.2 and 7.2 but
at 6.8 and 4.1 instead. To clarify which of the plasma enzymes derive from the kidney, the pH curve of the control subjects was subtracted from that of the anephric patients. The resulting differences are depicted in the right panel. For this calculation, the overlapping parts of the neighboring maxima had to be taken into consideration. Increasing enzyme levels are positively, and decreasing levels are negatively, plotted. The resultant activities for the pH optima at 4.1 and 5.3 are clearly augmented. On the other hand, the peak at 6.2 (pH optimum of renal renin) disappeared almost completely, and the enzyme values at 7.2 remained constant.

**Discussion**

The identification and quantification of Ang I-forming angiotensinogenases in extrarenal vasculature was a prerequisite for the present study. Renin antibodies were not used for measurement of Ang I-forming angiotensinogenase activities because it is unknown whether they react with the extrarenal vascular enzymes. Molecular biological techniques for the identification of renal renin were not used for the same reason. In addition, these analytical methods cannot differentiate between the multiple forms of renin or reninlike enzymes presumably present in human extrarenal vasculature, as has been demonstrated for the rat. Ang I-forming angiotensinogenase activities were thus assayed by enzyme-kinetically measuring the initial Ang I formation rate with purified natural angiotensinogen. Linearity of the kinetics shown in Figure 1 indicates that the analytical procedure was reliable. Human plasma renin consumes sheep angiotensinogen faster than the homologous substrate. This is also the case for human extrarenal vascular Ang I-forming angiotensinogenases. The four pH optima found for the reaction of extrarenal vascular Ang I-forming angiotensinogenases with sheep or human angiotensinogens indicate the existence of four different enzymes. However, recent studies in the rat showed that one pH optimum may represent several enzymes, which may possibly differ in their carbohydrate contents, as previously assumed by Sessler et al. The biochemical relation of extrare-
The main aim of this study was to examine the theory of Jiménez Díaz et al., which postulates the existence of renin or reninlike enzymes in human extrarenal arterial walls and their participation in pathogenesis of essential hypertension. The feasibility of this investigation mainly depended on the availability of suitable vascular samples. Because blood vessels from different regions had to be used initially, the distributions of Ang I-forming angiotensinogenases in different vascular tissues were measured. Consistent with results obtained earlier for the rat, highest Ang I-forming angiotensinogenase levels were found in the heart. This observation can be explained by the fact that specific Ang I-forming angiotensinogenase activities increase with decreasing vascular diameters and that numerous small blood vessels are present in cardiac tissue. The values obtained for (whole) arteries were slightly lower than those for carotid artery intima; this finding suggests luminal localization of the enzymes. The venous activities derive exclusively from varicose blood vessels and indicate that vascular Ang I-forming angiotensinogenases are suppressed in these pathological tissues. Likewise, the activities of umbilical cord vasculature are significantly lower than those of other blood vessels. Ang I-forming angiotensinogenase (pH optimum 5.4), angiotensin converting enzyme, and Ang I-degrading enzyme levels were determined simultaneously. Obviously, Ang II concentration is low in these arteries, when compared with others, because Ang I and Ang II formations are inhibited, while Ang I degradation is facilitated. The results suggest that in these blood vessels the vascular renin-angiotensin system scarcely affects fetal blood supply.

Although the existence of an extrarenal arterial Ang I-forming enzyme and its participation in essential hypertension has repeatedly been postulated, this hypothesis has not yet been confirmed in humans,
possibly because of the difficulty of obtaining sufficient amounts of suitable vasculature. Mainly, aortic sections and intraoperatively separated samples of carotid artery intima were available for our purpose. In carotid artery intima higher, but not significantly different, enzyme values than in whole arterial tissues were found. This may be caused by several factors. The Ang I-forming angiotensinogenases are probably concentrated in the luminal parts of the arteries. However, it is also possible that the enzymes mediate special physiological functions in the carotid artery and thus are present there in higher concentrations.

In essential hypertension, extrarenal arterial Ang I-forming angiotensinogenases are clearly not stimulated as previously postulated although there is a slight indication of greater scatter in the hypertensive patients than in the control subjects. Since in this disease plasma renin activities may be high, normal, or low, we wanted to establish whether the vascular enzymes behave similarly. The correlation coefficients and probabilities for the vascular Ang I-forming angiotensinogenases (pH optimum 5.1 and 4.0) and plasma renin were thus evaluated. The data obtained suggest the existence of subgroups of essential hypertensive patients with high, normal, and low extrarenal Ang I-forming angiotensinogenase activities and common controlling mechanisms for vascular Ang I-forming angiotensinogenases and plasma renin. Because extrarenal arterial Ang I-forming angiotensinogenases are generally stimulated in spontaneously hypertensive rats, these animals can only be assumed to be a model for that subgroup of essential hypertension with elevated vascular Ang I-forming angiotensinogenase activities.

Finally, we wanted to establish whether the Ang I-forming angiotensinogenases in extrarenal vasculature are demonstrable in plasma. For this purpose, the effect of pH on plasma Ang I-forming angiotensinogenase activities was studied. Two major and two minor pH peaks were found. Clearly, bilateral nephrectomy affected the plasma enzymes. A significant diminution was observed at pH 6.2, which indicated an enzyme derived from the kidney. However, the heights of two other pH optima (at 4.0 and 5.3) increased distinctly. Ang I-forming angiotensinogenases with the same pH optima are contained in extrarenal arterial walls and are also present in the kidney. However, in renal extract the vascular pH optimum is superimposed by that of the main form of kidney renin. Initial investigations performed by fast protein liquid chromatography confirm this assumption (Figure 2). Although bilateral nephrectomy should reduce the activities of those vascular Ang I-forming angiotensinogenases released by the kidney, such a diminution was not observed. Therefore, we assume that removal of the kidneys causes an effective stimulation of these enzymes, possibly due to the drastic reduction of plasma renin activity.

The experimental results obtained indicate the existence of an intrinsic human vascular renin-angiotensin system also contained in the kidney and heart and interrelated with the plasma system. The postulate of its general stimulation in essential hypertension could not be confirmed.

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References
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