Studies on Angiotensinogen of Plasma and Cerebrospinal Fluid in Normal and Hypertensive Human Subjects

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SUMMARY The presence of a renin-angiotensin system in the central nervous system (CNS) has been demonstrated by several investigators, but little is known regarding the origin of its components. In this study we have compared the immunological and physical-chemical nature of angiotensinogen in plasma and cerebrospinal fluid (CSF) of human subjects and explored whether differences are present in CSF angiotensinogen concentrations of normal and hypertensive subjects. No significant differences in the nature of plasma and CSF angiotensinogen was observed with respect to molecular weight (65-70,000) electrophoretic mobility ($R_{eq} = 0.67 \pm 0.003$) or angiotensin I (AI) generated (pl = 6.6). Following isoelectric focusing, the plasma angiotensinogen was shown to consist of a single component with an isoionic point of 4.40 ± 0.04. CSF angiotensinogen, on the other hand, resolved into three components (pl = 4.76 ± 0.02; 5.16 ± 0.04; 5.76 ± 0.04).

Although no correlations were observed between angiotensinogen levels in the CSF or plasma with blood pressure (BP), a statistically significant difference in angiotensinogen concentration of both plasma and CSF was observed between normotensive and hypertensive subjects. The differences in the chemical and immunological nature of human plasma and CSF angiotensinogens suggest that the angiotensinogen of CSF is not of peripheral origin. (Hypertension 2: 432-436, 1980)

KEY WORDS • angiotensinogen • cerebrospinal fluid • isoelectric focusing

Involvement of the renin-angiotensin system in the initiation and maintenance of hypertension has received considerable attention. To date, however, no unifying hypothesis for the participation of the peripheral renin-angiotensin system and hypertension has evolved. Recent studies have indicated that this enzyme system is present in the central nervous system (CNS) and as such may influence blood pressure (BP). Whether the components of the peripheral system cross the blood-brain barrier are locally synthesized is not clear. Renin and other enzymes with renin-like activity have been identified in various regions of the CNS by several investigators, and the presence of angiotensinogen in the CNS has been demonstrated. Morris and Reid have recently shown angiotensinogen to be located mainly in extracellular fluid since insignificant quantities are present in the membrane fraction of dog mid- and forebrain. Comparisons of the physical characteristics of angiotensinogen from plasma and cerebrospinal (CSF) fluid indicated similarities between the two proteins, and it has been suggested that plasma angiotensinogen may cross the blood-CSF barrier. It has also been shown, however, that alterations in plasma angiotensinogen, caused either by nephrectomy or administration of drugs known to increase plasma angiotensinogen, failed to influence the levels of angiotensinogen in CSF.

In this study, we have compared several physical-chemical parameters and the immunological nature of angiotensinogen of plasma and CSF obtained from both normal and hypertensive human subjects.

Material and Methods

Clinical Material

We studied patients with hypertension, ranging from mild to severe (n = 23); the majority were receiving antihypertensive treatment. Normotensive subjects (n = 19) were undergoing orthopedic surgery and were of comparable age and without overt and significant systemic or CNS disease.

Venous blood and CSF samples were obtained from male patients given spinal anesthesia before undergoing surgery and from patients of the Neurology Service undergoing clinical tests requiring lumbar punctures. This study includes patients from the Japanese Defense Forces Central Hospital, Tokyo, Japan.
ANGIOTENSINOGEN OF PLASMA AND CSF

Angiotensinogen Measurements

Angiotensinogen levels were determined by incubation of plasma or CSF with added homologous renin in the presence of angiotensinase inhibitors.7,8 A direct radioimmunoassay developed for plasma angiotensinogen was also utilized as previously described;9 no interference was evident when this assay was employed using CSF samples.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel (7%) electrophoresis was performed at pH 8.6 as previously described using the albumin front for determination of Rf values.9

Molecular Weight

Molecular weights were determined by gel exclusion chromatography using AcA 44 (LKB) as the support medium on a 1.6 X 100 cm column equilibrated in 0.05 M Tris HCl, pH 8.6, using a previously described method.10

Isoelectric Focusing

Plasma and CSF samples (0.1 ml) were applied directly to isoelectric focusing gels containing pH 3-10 ampholines according to the method of Allen and Mauer.11 Gels were sliced at 2 mm intervals on a Gilson gel slicer. Following measurements of the pH of each slice, the pH was adjusted with 1M sodium phosphate buffer to pH 7.4 and incubated with renin for angiotensinogen determination. Results are referred to as isoionic point when samples were not dialyzed against distilled deionized water prior to electrophoresing.

All data are given with the standard error;12 sialic acid groups were cleaved with neuraminidase.5

Results

Using measurement of Al generated upon incubation with homologous kidney renin as the method of quantitation, angiotensinogen levels from unselected human subjects were 3585 ± 162 ng AI equivalents/ml for plasma and 369 ± 31 for CSF (n = 42). A direct radioimmunoassay for plasma angiotensinogen, which gives equivalent results in normal and essential hypertensive subjects, was also employed.9 With this method, only 2.1 ± 0.8 ng AI equivalents/ml (n = 49) were detected in CSF, indicating an immunological difference between plasma and CSF angiotensinogens. By the direct assay, CSF angiotensinogen represents only 0.08% of the plasma concentration, whereas by AI generation, CSF angiotensinogen reflects 10.3% of the plasma concentration. Angiotensinogen determinations obtained from a selected group of normal and hypertensive subjects are shown in table 1. In a group of nine normotensive patients with concomitant systemic disease, the direct assay yielded significantly elevated angiotensinogen levels in CSF with respect to normal or hypertensive subjects (5.1 ± 1.4 ng/ml, p < 0.05).

Table 1. Plasma and CSF Angiotensinogen Levels

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Hypertensive</th>
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<tbody>
<tr>
<td>Plasma</td>
<td>3504 ± 290</td>
<td>4126 ± 190</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>(n = 19)</td>
<td></td>
</tr>
<tr>
<td>CSF*</td>
<td>294 ± 56</td>
<td>394 ± 55</td>
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<tr>
<td>(n = 8)</td>
<td>(n = 19)</td>
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<tr>
<td>CSF†</td>
<td>1.0 ± 0.3</td>
<td>1.9 ± 0.3</td>
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<tr>
<td>(n = 10)</td>
<td>(n = 18)</td>
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</table>

* Determined by AI generation.
† Determined by direct radioimmunoassay.
| Significance level between angiotensinogen levels between normal and hypertensive subjects.

Incubation of plasma and CSF pools with neuraminidase, to remove sialic acid residues, had no statistically significant effect on the concentration of angiotensinogen by AI generation. Using the direct angiotensinogen assay, the same experiment yielded a less than 1% decrease in plasma and CSF angiotensinogens upon exposure to neuraminidase.

Although immunological differences were evident between angiotensinogen in human plasma and CSF, several physical-chemical characteristics indicated similarities. Molecular weights, determined by gel exclusion chromatography were 65–70,000 for both plasma and CSF angiotensinogen with only a single molecular weight component evident (fig. 1). Electrophoretic mobility in polyacrylamide gels at pH 8.6, indicated an Rf = 0.67 ± 0.003 for both plasma and CSF angiotensinogen with respect to the migration of albumin (fig. 2). Again, only a single form of angiotensinogen was evident by this procedure.

When plasma and CSF were incubated with excess homologous renal renin, the isoionic point of the

![Figure 1. Gel exclusion chromatography with Ultrogel AcA 44.](http://hyper.ahajournals.org/)

OPTICAL DENSITY (280nm) vs. ANGIOTENSINOGEN (ng AI equiv/ml) vs. ELUTION VOLUME (mL)

- Represents plasma.
- Represents cerebral spinal fluid angiotensinogen concentrations; the solid line represents optical density measurements of plasma.
FIGURE 2. Polyacrylamide gel electrophoresis (pH 8.6) of plasma and CSF angiotensinogen.

generated product, as detected by radioimmunoassay for AI, was observed at pH 6.6, identical to that of synthetic AI. The pressor response to the generated material of these two samples was also not distinguishable from AI in a pentolitium ganglionic blocked rat.

Although the molecular weight, electrophoretic mobility and nature of the generated end product of plasma and CSF angiotensinogen were identical, clear distinctions in the isionic points and distribution patterns of plasma and CSF angiotensinogens were apparent following isoelectric focusing (fig. 3 and table 2). Multiple forms of angiotensinogen were seen by angiotensin I generation in CSF of both normal and hypertensive subjects. However, no statistically significant differences were observed in the isionic points of these multiple forms of normotensive or hypertensive subjects. To determine if sialic acid groups contributed to the multiple forms of angiotensinogen in CSF observed following isoelectric focusing, CSF was treated with neuraminidase. The isoelectric points shifted from 5.0, 5.5 and 6.2 to a single form at pH 6.4.

No linear correlation was observed between the plasma and CSF angiotensinogen concentrations in

<table>
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<th>TABLE 2. Plasma and CSF Isoionic Points of Angiotensinogen</th>
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<td>(ng AI equivalents/ml)</td>
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<tr>
<td>Normotensive</td>
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<tr>
<td>Hypertensive</td>
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<tr>
<td>Plasma 4.40 ± 0.04 (n = 4)</td>
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<tr>
<td>4.43 ± 0.03 (n = 4)</td>
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<tr>
<td>CSF 4.80 ± 0.03 (n = 6)</td>
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<tr>
<td>4.76 ± 0.02 (n = 5)*</td>
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<tr>
<td>5.23 ± 0.03</td>
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<tr>
<td>5.16 ± 0.04</td>
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<tr>
<td>5.75 ± 0.03</td>
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<td>5.76 ± 0.04</td>
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* A malignant hypertensive patient in addition had angiotensinogen forms with isionic points at pH 3.0, 3.3, 3.9, 4.1, 5.9, 6.1 and 6.9.

FIGURE 3. Upper: Typical isoelectric focusing pattern of CSF angiotensinogen of either a normotensive or hypertensive subject. Lower: Pattern of a subject with malignant hypertension and cerebrovascular accident.
either the normal or hypertensive subjects (table 3). Although statistically significant differences were observed between the concentration of plasma and CSF angiotensinogen in normal and hypertensive subjects (table 1), no linear correlations were observed when plasma and CSF angiotensinogen levels were compared with mean or systolic blood pressure.

**Discussion**

The similarity of human plasma and CSF angiotensinogen with respect to their molecular weights and electrophoretic mobility is in agreement with results of other investigators using dogs and sheep. Identification of the peptides generated following the addition of renin as demonstrated by both identical isoionic points of these peptides with synthetic angiotensin I and their biological response in the rat also indicate similarities of angiotensinogen of plasma and CSF. In contrast to these results showing similarities of plasma and CSF angiotensinogen, the immunological studies indicated structural differences in the two forms of angiotensinogen. We have previously reported several forms of angiotensinogen in the plasma of women on oral contraceptives which are also not recognized by the antibody specific for the major form of plasma angiotensinogen. However, the $R_{fAib}$ values of these proteins from women on oral contraceptives were 0.16 and 0.34 on polyacrylamide gel electrophoresis, different from that found in CSF. The CSF angiotensinogen migrates as a single component with an $R_{fAib} = 0.67$, identical to that of the major form of angiotensinogen in plasma that is recognized by the antiserum. This result indicates that the angiotensinogen of CSF may not be of plasma origin and may possibly be locally synthesized. Further evidence in support of a locally synthesized angiotensinogen is the non-identity of their isoionic points (table 2). Whether the angiotensinogens found in CSF are structurally similar to those of plasma or whether they represent an entirely different protein, possibly locally synthesized, from which angiotensin I can be cleaved by renin will have to await further purification and characterization of the CSF forms.

Exposure of CSF to neuraminidase demonstrated the glycoprotein nature of angiotensinogen and indicated that sialic acid groups prominently contribute to the multiplicity of isoionic points observed. The observed difference in isoionic point (nondialyzed samples) and isoelectric points (dialyzed samples) we observe in CSF are in agreement with the differences observed by Faier et al. in human plasma. The effect of neuraminidase to shift the isoelectric points to a higher pH are in agreement with those of Printz et al. These investigators demonstrated that removal of sialic acid from angiotensinogen of sheep CSF, resulted in an increase in pi and a loss of the multiple forms. Although the sialic acid groups influence the observed isoelectric points, their removal from CSF angiotensinogen by neuraminidase treatment did not alter the fact that these proteins remained immunologically distinct from the plasma angiotensinogen and indicate further distinctions between the physical and chemical nature of plasma and CSF angiotensinogens.

At the present time, data are not available to allow for an estimation of whether all substrate forms in CSF show a small percent of cross reactivity with our specific antiserum or whether a specific form cross reacts. The sialic acid components of CSF and plasma angiotensinogen may be responsible for the high molecular weight we previously reported utilizing Sephadex G 150 as the support medium. Interactions of sialic acid groups and Sephadex have been reported. The molecular weight of 65,000 we have obtained, for angiotensinogen in both CSF and plasma, using AcA 44, is in agreement with a previous report of the molecular weight of human plasma angiotensinogen as determined by centrifugation technics.

With the exception of a single patient with malignant hypertension, where several additional forms of angiotensinogen were evident in the CSF, our results indicate similar isoionic points for the multiple angiotensinogens in normal and hypertensive subjects. Preliminary results indicate however, that the concentration of the various forms of angiotensinogen in CSF may differ in normal and hypertensive subjects.

The concentration of angiotensinogen we observe in the CSF by angiotensin I generation is equivalent to that observed by Printz et al. and represents 10.3% of the plasma level. These data indicate a selective enrichment of human CSF angiotensinogen when compared with plasma on a mg protein per ml basis as has been reported in both sheep and dog CSF, and again speak against a plasma origin for the protein. A lack of penetration of plasma angiotensinogen into the CSF would tend to support the conclusions of Reid and Day who demonstrated that a twofold increase in plasma angiotensinogen in the dog, induced by either nephrectomy or administration of dexamethasone, did not raise the CSF levels.

In conclusion, this study has demonstrated differences in the physical and chemical composition and immunological nature of angiotensinogen of plasma and CSF suggesting a nonperipheral origin. We have also shown statistically significant differences to occur in the levels of CSF angiotensinogen in normal and hypertensive human subjects. Whether or not these elevated levels, in the central nervous system, contribute to the maintenance of hypertension remains to be determined.

<table>
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<tr>
<th>TABLE 3. Linear Correlation Coefficients of Plasma and CSF Angiotensinogen Levels in Normal (n = 8) and Hypertensive Subjects (n = 18)</th>
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<td>-----------------------------------------------</td>
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<tr>
<td>Plasma vs CSF*</td>
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<tr>
<td>Plasma vs CSF†</td>
</tr>
<tr>
<td>CSF* vs CSF†</td>
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</tbody>
</table>

* Determined by angiotensinogen generation.  † Determined by direct radioimmunounassy.
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