studies using clinical and/or experimental approaches will address this hypothesis, regarding which little information is currently available. Similarly, we have no useful information at present regarding possible interactions between S100B and sex hormones, which could also be hypothesized on the basis of the gender differences found in S100B concentrations. More generally, the growing body of evidence that indicates a biological role of S100B as a cytokine points to the usefulness of future studies on possible interactions between this protein and individual hormonal patterns.

The most probable origin of S100B in peripheral blood, as previously reported in several studies, is nervous tissue, although we cannot exclude the possibility that it may also be released from other sites of concentration, such as adipose tissue (20). However, data on the presence of the protein in adipose tissue at the ages studied here are not conclusive.

Finally, the different peaks of protein concentration in the two sexes could, in common with other clinical and anthropometric studies (i.e., height/weight growth reference curves), suggest the possibility that brain maturation in the pediatric period differs in males and females, as it does in the intrauterine and adult periods (9, 21). In this respect it could be relevant that genetically modulated overexpression of S100B has been reported to affect the behavior of female mice without causing any appreciable effects in males in experimental models (22).

In conclusion, the reference curve for S100B protein in peripheral blood in healthy pediatric patients constitutes a useful tool to evaluate pathologic alterations of the protein during this period and also suggests an approach for future investigations into the suggested neurotrophic role of the protein, which could potentially be related to the process of maturation, including hormone concentrations.

References


Quantitative Spectrophotometric Microplate Assay for Angiotensin-converting Enzyme in Cerebrospinal Fluid, J. Alan Erickson,1,* Roxann Cousin,2 James T. Wu,1,3 and Edward R. Ashwood1,3 (* ARUP Institute for Clinical and Experimental Pathology, LLC, Salt Lake City, UT 84108; ARUP Laboratories, Special Chemistry Section, Salt Lake City, UT 84108; Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT 84132; address correspondence to this author at: ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108; fax 801-584-5109, e-mail ericksja@aruplab.com)

Angiotensin-converting enzyme (ACE; EC 3.4.15.1) catalyzes the formation of angiotensin II by cleaving the C-terminal histidyl-leucine dipeptide from angiotensin I (1). Indications are that ACE is affiliated with an autonomous renin-angiotensin system of the brain that participates in physiologic processes inside the brain (2, 3). In addition, studies suggest that changes in ACE concentrations in brain tissue, caused by various neurologic disorders, are reflected by alterations in ACE activity in cerebrospinal fluid (CSF) (4). For example, increased ACE concentrations in CSF are associated with neurosarcoidosis (4-7), with affected patients generally having activities approximately twofold or more higher than those of healthy individuals (4, 6, 7). Increased CSF ACE has also been implicated in neurologic diseases, such as bacterial and viral meningitis and Behcet disease (4-7). Decreased concentrations have been reported in patients with Alzheimer disease, Parkinson disease, and progressive supranuclear palsy (8, 9).
The spectrophotometric assays customarily used for serum ACE lack the sensitivity for measuring the concentrations typically found in CSF. Consequently, more sensitive and costly methodologies are routinely used, such as fluorometric assays or HPLC (4).

To provide a more economic assay to measure CSF ACE for the screening of neurosarcoïdosis, several attempts were made at modifying commercially available reagents and protocols used specifically for spectrophotometric measurement of serum ACE. A successful assay was eventually established that utilizes the ability of ACE to hydrolyze the tripeptide N-[3-(2-furyl)acyrloyl]-L-phenylalanylglucylglycine to furylacyrloylphenylalanine and glycyglycine (10). However, the sample volume (0.6 mL) required to achieve acceptable sensitivity was impractical. The assay was therefore reformatted for a 96-well microplate. This dramatically decreased sample and reagent volumes and increased accuracy by making duplicate measurements viable for practically all specimens received in our laboratory.

ACE reagent, calibrator, bovine serum albumin (BSA), and buffer reagents were purchased from Sigma Diagnostics®. Immulon 1B Removewell® Strips, holders, and plate sealers were purchased from Dynex Technologies, Inc. The SPECTRAmax® PLUS plate reader was manufactured by Molecular Devices Corp. and was controlled with their ProMax software. Data analysis was performed using Microsoft Excel software.

CSF was collected in plastic tubes and stored at 2–8 °C for 7 days or less, or was frozen at −20 or −70 °C for longer storage. Severely hemolytic or xanthrochromic specimens were avoided.

ACE reagent was reconstituted at 0.6 times the manufacturer’s recommended volume. ACE calibrator was reconstituted as instructed; diluted with 10 g/L BSA in phosphate-buffered saline (PBS; 0.008 mol/L Na2HPO4, 0.003 mol/L KH2PO4, 0.150 mol/L NaCl; pH 7.2) to activity values of 5.0, 3.0, and 1.0 U/L; and aliquoted for fluorometric assays or HPLC. Mean corrected calibrator ΔA was 3.9, and 5.6 U/L, respectively, for the two specimens at 1.0 and 1.6 U/L, respectively. All samples showed marked interference, producing activities of 2.0, 3.0, and 4.8 U/L and 2.9, 3.9, and 5.6 U/L, respectively, for the two specimens at the three added volumes.

A split-sample study (n = 35) against a reputable assay (Roche FARA) used by the University of Washington was completed. Deming regression generated a slope and y-intercept of 0.9684 and 0.1466 U/L, respectively (R² = 0.938). Note, however, that the results from the university are reported in whole numbers with a detection limit of 1 U/L. Activities from our assay are to the nearest 0.1 U/L because of the higher sensitivity.

Measurement of 167 CSF specimens, deemed normal from negative oligoclonal banding and myelin basic protein results, produced an upper limit of the reference interval of 2.5 U/L at the 95% confidence limit [mean (SD), 1.2 (0.63) U/L]. Only four (2.4%) of the results exceeded the calculated reference interval (Fig. 1). Analyses were also made vs patient age (8–79 years) and gender. As shown in Fig. 1, no correlation between normal CSF ACE activity and patient age was evident. The same was true when activities were segregated by gender, with males (n = 63) and females (n = 104) generating upper limits of the respective reference intervals of 2.4 and 2.5 U/L.

As described previously, severe hemolysis may artificially increase CSF ACE measurements. This especially...
becomes relevant if the patient has highly increased blood ACE. Clean taps are therefore preferred. However, only two specimens were immediately excluded from our reference interval study, and only one was excluded from the correlation study because of visibly severe contamination. No major discrepancies or excessive outliers were evident in the data derived from the remaining samples, some of which did exhibit minor contamination. In other words, the majority of the CSF specimens received in our laboratory are sufficiently clean for analysis using the assay. Nevertheless, we recommend that any visible contamination be noted in case of questionable results and that grossly hemolyzed specimens be avoided altogether. In addition, hemolyzed CSF that has been centrifuged may contain residual serum that could possibly influence results.

The use of our assay and reference interval for the screening of neurosarcoidosis parallels other published studies addressing the disease. As stated previously, affected patients frequently have CSF ACE activities approximately twofold (or more) higher than those in healthy individuals. For example, in a study by Schweisfurth and Schöberg-Schiegnitz (4), utilizing a fluorometric assay, patients with active sarcoidosis of the brain had a median CSF ACE activity of 88.5 U/mL compared with a median control patient value of 39.5 U/mL. Oksanen et al. (6), using a radioactive inhibitor binding assay, showed that patients with active neurosarcoidosis had a mean CSF ACE activity of 1.42 U/mL compared with a control value of 0.78 U/mL. Another study by Jones et al. (7), involving two patients diagnosed with neurosarcoidosis, listed CSF ACE activities of 1.8 and 5.4 μmol·L⁻¹·min⁻¹ for two affected patients vs a mean value of 0.59·L⁻¹·min⁻¹ for 38 control individuals. The upper limit of our reference interval (2.5 U/L) and the mean for our reference population (1.2 U/L) retain an analogous relationship, mirroring the approximate twofold or greater increases disclosed above.

In summary, the spectrophotometric microplate-formatted CSF ACE assay described here has adequate sensitivity, accuracy, precision, and reliability for the screening of neurosarcoidosis. The assay incorporates economical and available commercial reagents and common buffers in a configuration that allows for a reasonable CSF sample volume in addition to use of instrumentation typically found in the clinical laboratory. By measuring 167 CSF specimens from healthy controls, we have established an upper reference value of 2.5 U/L. A single reference interval is reasonable because no relationship between ACE activities and patient age or gender is evident. In addition, the assay adequately correlates with an established assay used by the University of Washington despite that assay’s limitations. Satisfactory correlation is also supported by the fact that our upper reference value varies by only 0.5 U/L (2.5 vs 2 U/L), a difference that is irrelevant considering the sensitivity issues addressed previously. It does, however, provide additional assurance that our assay is working within the parameters of its intended purpose. Furthermore, our results are analogous to those obtained in other studies addressing CSF ACE activity in neurosarcoidosis screening.

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References


