Cerebrospinal Fluid Atrial Natriuretic Factor in Intracranial Disease

Michael N. Diringer, MD, Jeffrey R. Kirsch, MD, Paul W. Ladenson, MD, Cecil Borel, MD, and Daniel F. Hanley, MD

We tested the hypothesis that the concentration of atrial natriuretic factor in the cerebrospinal fluid is an indicator of brain injury in patients with intracranial disease. Atrial natriuretic factor concentration was measured in 72 samples of cerebrospinal fluid from 28 patients with intraventricular drains and in nine samples from outpatient controls undergoing diagnostic lumbar puncture. Levels were correlated with diagnosis; systemic fluid administration; concentration of atrial natriuretic factor in the plasma; intracranial pressure; sodium, glucose, and protein concentrations, osmolality, and cell count in the cerebrospinal fluid; sodium concentration in the serum; and hemodynamics. Atrial natriuretic factor concentration was highest in cerebrospinal fluid from patients with intracerebral hematoma, followed by those with obstructive hydrocephalus and subarachnoid hemorrhage (19±2, 13±3, and 8±2 pg/ml, respectively); atrial natriuretic factor concentration was <4 pg/ml in the controls. Patients treated with fluid restriction had significantly higher atrial natriuretic factor levels than those receiving maintenance or high-volume fluids (16±3, 8±2, 10±1 pg/ml, respectively). The concentration of atrial natriuretic factor in the plasma was significantly elevated in patients with intracerebral hematoma and subarachnoid hemorrhage (155±38 and 92±20 pg/ml, respectively) and did not correlate with fluid administration or the concentration of atrial natriuretic factor in the cerebrospinal fluid. Neither cerebrospinal fluid nor plasma concentrations of atrial natriuretic factor correlated with intracranial pressure; cerebrospinal fluid sodium, glucose, or protein concentrations, osmolality, or cell count; serum sodium concentration; or hemodynamics. We conclude that the concentration of atrial natriuretic factor in the cerebrospinal fluid is a nonspecific indicator of brain injury. Cerebrospinal fluid levels of atrial natriuretic factor may be elevated in response to dehydration therapy. Elevated plasma levels of atrial natriuretic factor suggest a central mechanism modulating cardiac release. (Stroke 1990;21:1550–1554)

Atrial natriuretic factor (ANF) was first recognized as a peptide produced by atrial myocytes involved in the regulation of body sodium and intravascular volume and has subsequently been identified in the central nervous system (CNS). A group of hypothalamic neurons contain ANF in their cell bodies and nerve terminals, and ANF receptors are located in circumventricular structures, the area postrema, and the choroid plexus. Like peripheral nervous system ANF, CNS ANF appears to contribute to the regulation of body sodium and intravascular volume. Intracerebroventricular administration of ANF produces natriuresis and diuresis; CNS-administered ANF inhibits the release of arginine vasopressin and the intake of salt and water. High sodium intake reduces the brain ANF content, and dehydration reduces the hypothalamic ANF content in rats.

Like other CNS peptides, ANF in the cerebrospinal fluid (CSF) appears to originate in the CNS. Intravenous infusions of ANF do not alter its concentration in the CSF, and labeled ANF infused into the carotid artery does not enter the choroid plexus. Disturbances of body sodium and intravascular volume regulation are common following aneurysmal subarachnoid hemorrhage (SAH). An association between hyponatremia, hypovolemia, and salt wasting has been described in SAH, but the pathogenesis of the disturbance has not been established. Previous reports did not demonstrate a link between...
were collected from patients who were undergoing continuous CSF drainage. Six CSF samples were investigated. Therefore, we tested the hypothesis that the CSF concentration of ANF is an indicator of brain injury in patients with intracranial disease. We compared CSF ANF concentrations in patients with SAH, hydrocephalus, and intracerebral hematoma (ICH) to those in outpatients having diagnostic lumbar puncture. In addition, we correlated CSF ANF concentration with the volume of therapeutic fluid administered, the plasma ANF concentration, serum sodium concentrations, CSF and serum osmolality, CSF glucose and protein concentrations, intracranial pressure, and hemodynamics.

Subjects and Methods

Seventy-two samples of ventricular CSF were collected from 28 patients admitted to the Neurosciences Critical Care Unit of the Johns Hopkins Hospital. The patients had an intraventricular catheter placed for the treatment of hydrocephalus or the monitoring of intracranial pressure. Diagnoses included SAH, obstructive hydrocephalus, and ICH. Patients with SAH were classified as low-grade (Hunt and Hess grades 1 and 2) or high-grade (Hunt and Hess grades 3–5). Nine CSF samples were collected from outpatients undergoing diagnostic lumbar puncture.

Intracranial pressure was continuously monitored in all patients, and CSF was drained if intracranial pressure was >10–20 mm Hg. Samples were obtained from CSF specimens routinely collected to monitor for infection. When volume permitted, CSF osmolality, sodium, glucose, and protein concentrations, and cell count were determined from the same specimen. The duration of CSF drainage prior to specimen collection was classified as 0, <2 hours, 2–24 hours, or >24 hours. Plasma ANF concentration, serum sodium concentration, serum osmolality, intracranial pressure, heart rate and rhythm, blood pressure, and, when available, central venous pressure and pulmonary capillary wedge pressure were measured simultaneously. The majority of specimens were collected from patients who were undergoing continuous CSF drainage. Six CSF samples were collected immediately after the insertion of an intraventricular catheter and the remainder were collected after intracranial pressure was stable for at least 2 hours. Level of fluid administration for the 24 hours prior to specimen collection was classified as fluid-restricted (<1.5 l/day), maintenance fluids (1.5–3 l/day), or high-volume fluids (>3 l/day) for rehydration or prophylactic volume expansion following SAH. In 15 patients additional CSF samples were collected 2–10 days after the initial specimen. In eight SAH patients sodium concentration was measured in a 24-hour urine specimen collected on the day of CSF sampling. Glasgow Coma Scale score was determined daily.

Osmolality, sodium and protein concentrations, and cell count in the CSF were determined in the hospital's clinical laboratory using standard methods. CSF samples were collected in plain glass tubes, and plasma samples were collected in glass tubes containing ethylenediaminetetraacetic acid and 1 µg/ml pentastin A. Specimens were cooled immediately to 4°C, separated by centrifugation at 2,000g for 10 minutes at 5°C, and then stored at −70°C. Specimens were thawed immediately before extraction and assay.

Before assay, CSF (2.5 ml) and plasma (1.25 ml) samples were acidified with 2.5 ml 0.1% trifluoroacetic acid (TFA) and centrifuged at 2,000g for 25 minutes at 5°C. ANF was extracted from both CSF and plasma by loading the supernatant onto preactivated (5 ml 60% acetonitrile, 0.1% TFA) C18 octadecyl silica cartridges (Sep-Pak C18, Waters Assoc., Milford, Mass.), which were then washed with 20 ml 0.1% TFA and eluted with 3 ml 60% acetonitrile, 0.1% TFA solution. Extracted samples were then dried in a centrifugal concentrator (Spin-Vac, Farmingdale, N.Y.) with a liquid nitrogen coolant and resuspended in 250 µl assay buffer for analysis in duplicate. ANF concentration was quantified by specific radioimmunoassay for human α-ANF (Peninsula Laboratories, Belmont, Calif.). Assay sensitivity was 4–8 pg/tube; intra-assay and interassay coefficients of variation were 5% and 19%, respectively. In our laboratory, mean±2 SD plasma ANF concentration in 38 normal volunteers was 21±16 pg/ml.16

To determine recovery, synthetic human α-ANF (Peninsula Laboratories) was added before extraction to plasma samples (n=10) and three types of CSF samples: 1) clear CSF with a protein concentration of <45 mg/dl (n=8), 2) yellow CSF with a protein concentration of >200 mg/dl (n=6), and 3) CSF with visual evidence of hemolysis (n=9). Synthetic α-ANF was added to raise the final concentration in plasma from 26 to 102 pg/ml and in CSF from 6 to 26 pg/ml. Mean±SEM recovery of synthetic α-ANF from plasma was 83±3%. Recovery did not differ significantly among clear (82±6%), yellow (84±3%), and hemolyzed (77±9%) CSF samples. Results are expressed as raw values not corrected for extraction.

For comparisons among patients grouped by diagnosis and SAH grade, only the initial CSF specimen was used. When analyzing physiologic variables, all samples for each patient were included. Since the data...
TABLE 1. Diagnoses in Patient Population and Controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Diagnosis</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subarachnoid hemorrhage</td>
<td>Acute aneurysmal subarachnoid hemorrhage</td>
<td>13</td>
</tr>
<tr>
<td>Obstructive hydrocephalus</td>
<td>Infratentorial arteriovenous malformation</td>
<td>3</td>
</tr>
<tr>
<td>Intracerebral hemorrhage</td>
<td>Infratentorial tumor</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Supratentorial tumor</td>
<td>2</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acute aneurysmal subarachnoid hemorrhage</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Infratentorial arteriovenous malformation</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Infratentorial tumor</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Supratentorial tumor</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Peripheral neuropathy</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Degenerative disease</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Rule out infection</td>
<td>5</td>
</tr>
</tbody>
</table>

were not normally distributed, they were analyzed by using nonparametric statistics. CSF and plasma ANF concentrations were compared among patients grouped by diagnosis and level of fluid administration and within patients over time by using the Kruskal-Wallis test. Within the SAH patients, comparisons between diagnoses, levels of fluid administration, and Hunt and Hess grades were made by using the Mann-Whitney test. Pearson's correlation was used to analyze the relations between CSF ANF concentration and duration of CSF drainage; CSF osmolality and sodium, glucose, and protein concentrations; serum osmolality and sodium and ANF concentrations; heart rate; blood pressure; central venous pressure; and pulmonary capillary wedge pressure. Results are expressed as mean±SEM. A probability value of less than 0.05 was considered significant.

Results

The 28 patients included 13 with SAH (five low-grade and eight high-grade), nine with obstructive hydrocephalus, and six with hypertensive ICH (Table 1). The nine controls were being evaluated for peripheral neuropathy, for degenerative disease, and to rule out infection. ANF was undetectable (<4 pg/ml) in the CSF of the controls; concentrations were significantly higher in the three patient groups (Figure 1). There was a significant difference in CSF ANF concentration between the ICH and SAH patient groups (Figure 1). Plasma ANF concentration was significantly higher than control in the SAH and ICH groups (Table 2), and the ICH group had a significantly higher plasma ANF concentration than the other two patient groups. Plasma ANF concentration did not correlate with CSF ANF concentration for any diagnosis. Within the SAH group, there was no difference in either CSF or plasma ANF concentration between low- and high-grade patients. Similarly, for the 15 patients with other diagnoses there was no relation between the Glasgow Coma Scale score and CSF ANF concentration.

There was a significant relation between CSF ANF concentration and the level of fluid administration. Patients who were fluid-restricted had a higher CSF ANF concentration (Figure 2) than those receiving maintenance or high-volume fluids. In the SAH group, all specimens were collected during the administration of high-volume fluids. In the hydrocephalus group, three specimens were collected during fluid restriction, 13 during the administration of maintenance fluids, and three during the administration of high-volume fluids. In the hydrocephalus group, three specimens were collected during fluid restriction, 13 during the administration of maintenance fluids, and three during the administration of high-volume fluids.

FIGURE 1. Scatter plot of cerebrospinal fluid (CSF) atrial natriuretic factor (ANF) concentrations in 28 patients by diagnosis and in nine controls. Each point represents single measurement. Horizontal line indicates mean. SAH, subarachnoid hemorrhage; HYDRO, obstructive hydrocephalus; ICH, intracerebral hematoma. *p<0.05 different from control; #p<0.05 different from SAH by Mann-Whitney test.

TABLE 2. Concentrations of Atrial Natriuretic Factor in CSF and Plasma by Diagnosis and Fluid Administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Diagnosis</th>
<th>n</th>
<th>CSF</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subarachnoid hemorrhage</td>
<td>13</td>
<td></td>
<td>8±2*</td>
<td>92±20*</td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>9</td>
<td></td>
<td>13±3*</td>
<td>39±9</td>
</tr>
<tr>
<td>Intracerebral hemorrhage</td>
<td>6</td>
<td></td>
<td>19±2*†</td>
<td>155±38*†</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>9</td>
<td>&lt;4</td>
<td>40±10</td>
</tr>
<tr>
<td></td>
<td>Fluid-restricted</td>
<td>7</td>
<td>16±3</td>
<td>114±61</td>
</tr>
<tr>
<td>Maintenance fluids</td>
<td>6</td>
<td></td>
<td>8±2§</td>
<td>87±65</td>
</tr>
<tr>
<td>High-volume fluids</td>
<td>35</td>
<td></td>
<td>10±1§</td>
<td>73±51</td>
</tr>
</tbody>
</table>

Values are mean±SEM pg/ml. *p<0.05 different from control by Kruskal-Wallis test. †p<0.05 different from subarachnoid hemorrhage and from subarachnoid hemorrhage and hydrocephalus, respectively, by Mann-Whitney test. §p<0.05 different from fluid-restricted by Kruskal-Wallis test.
tion of high-volume fluids. In the ICH group, seven specimens were collected during fluid restriction and one during the administration of maintenance fluids. Plasma ANF concentration did not differ among patients grouped by level of fluid administration (Table 2). CSF ANF concentration did not correlate with serum sodium concentration (131–151 meq/l), urinary sodium concentration (87–175 meq/l), heart rate (60–125 beats/min), central venous pressure (~5 to 11 mm Hg), or pulmonary capillary wedge pressure (5–20 mm Hg).

The CSF samples were collected over 2–10 days in 14 patients and for >3 weeks in one patient. Serial CSF ANF levels remained relatively stable for each patient (Figure 3). There was no significant difference between the mean initial and the mean final values (12.1±1.4 and 10.0±1.3 pg/ml, respectively). There was no relation between CSF ANF concentration and the duration of CSF drainage (1 hour to 10 days), CSF sodium concentration (136–164 meq/l), CSF osmolality (275–346 mosm), CSF glucose concentration (32–182 mg/dl), CSF protein concentration (9–280 mg/dl), CSF cell count (0–19,000 leukocytes and 13–>10⁶ erythrocytes), or intracranial pressure (~4 to 30 mm Hg).

In two patients CSF samples were obtained simultaneously from a ventricular catheter and by lumbar puncture. In the first patient, ANF concentrations were 19.6 and 17 pg/ml in the ventricular and lumbar CSF, respectively. In the second patient, ANF concentrations were 3 and 3.5 pg/ml in the ventricular and lumbar CSF, respectively.

Discussion

We tested the hypothesis that the CSF concentration of ANF is an indicator of brain injury, and we found that CSF ANF concentrations were elevated in patients with catastrophic intracranial disease. The ANF concentrations were highest in patients with ICH. For individual patients, serial ANF levels remained stable. The CSF but not the plasma ANF concentrations were elevated in patients receiving fluid-restricted therapy, but there was no relation to central venous pressure; pulmonary capillary wedge pressure; serum, urine, or CSF sodium concentration; serum or CSF osmolality; or intracranial pressure.

Hyponatremia is a frequent complication of SAH and has been attributed to excessive renal sodium and water losses. It has been suggested that this disturbance may be due to a "brain natriuretic hormone." Rosenfeld et al. found CSF ANF concentrations to be higher in patients with ruptured aneurysms than in those undergoing surgery for unruptured aneurysms. We found CSF ANF concentrations in SAH patients to be almost identical to those reported by Rosenfeld et al. However, we found higher concentrations in patients with hydrocephalus and ICH, intracranial diseases not regularly associated with sodium and volume loss. Therefore, it is doubtful that the elevation of CSF ANF concentrations represents a specific indicator of disturbed endogenous sodium and intravascular volume regulation in SAH. Rather, these data suggest that CSF ANF concentration may be a nonspecific indicator of CNS injury. It is possible that a more complex relation exists between CSF ANF concentration and sodium and intravascular volume regulation following SAH. This could be defined by concurrent measurements of intravascular volume, sodium balance, and fluid balance and other humoral regulators of sodium and intravascular volume.

The source of the additional ANF in the CSF of patients with intracranial disease has not been established. In normal animals, CSF ANF appears to originate in the CNS. It is unlikely that bleeding into the subarachnoid space or disruption of the blood–brain barrier could account for leakage of plasma ANF into the CSF because 1) there was no correlation between ANF concentrations in the CSF and plasma, 2) CSF ANF concentration was elevated in patients without hemorrhage, 3) ventricular CSF specimens were often uncontaminated by hemorrhage, and 4) CSF ANF concentration did not correlate with CSF protein concentration.
Unlike Doczi et al., we did not find a correlation between intracranial pressure and CSF ANF concentration. Samples in that study were collected at the time of intraventricular catheter insertion, whereas most of our samples were collected after a significant amount of CSF had been drained. To determine if CSF drainage could account for this difference, we correlated CSF ANF concentration with duration of drainage. We could not demonstrate a significant relation; however, the intervals used may have been too long to detect such an effect. We could not find a significant correlation between CSF ANF concentration and the Hunt and Hess grade. Suzuki et al. had previously reported that the CSF ANF concentration in patients with SAH did not correlate with the presence or absence of clinical vasospasm; however, those authors did not report the patients’ Hunt and Hess grades.

Plasma ANF concentrations were elevated in patients with SAH and ICH. The principal mechanism for controlling release of ANF from the heart is believed to be atrial stretch. However, in our patients plasma ANF concentration did not correlate with central venous pressure or pulmonary capillary wedge pressure, as has been previously reported in patients with SAH. These data suggest that neural mechanisms may influence the cardiac release of ANF, but there is no direct evidence to support this hypothesis.

We found elevated levels of CSF ANF in patients who were fluid-restricted or actively dehydrated. Higher concentrations of CSF ANF may reflect increased ANF release by hypothalamic neurons in an attempt to preserve intravascular volume. This is consistent with the observation that dehydration reduces the hypothalamic content of ANF. However, this finding must be interpreted with caution because different levels of fluid administration were not uniformly distributed among the diagnoses. Therefore, the differences in CSF ANF concentration due to diagnosis may confound the differences due to fluid administration. This relation should be more fully explored in carefully controlled studies.

In summary, we found CSF concentrations of ANF to be elevated in patients with ICH, obstructive hydrocephalus, and SAH. The CSF ANF levels were elevated in patients treated with dehydration therapy. The plasma ANF concentrations were elevated in patients with ICH and SAH. We conclude that the CSF ANF concentration is a nonspecific indicator of brain injury and that elevated levels may be seen during dehydration therapy. Elevated plasma ANF levels suggest a CNS mechanism modulating peripheral ANF release.

Acknowledgments

The authors wish to gratefully acknowledge the support of the nurses of the Neurosciences Critical Care Unit and the secretarial support of Ms. Yarvis Simmons.

References


Key Words: cerebrospinal fluid • hydrocephalus • natriuretic peptides, atrial • subarachnoid hemorrhage
Cerebrospinal fluid atrial natriuretic factor in intracranial disease.
M N Diringer, J R Kirsch, P W Ladenson, C Borel and D F Hanley

*Stroke.* 1990;21:1550-1554
doi: 10.1161/01.STR.21.11.1550
*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/21/11/1550

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org/subscriptions/