Cerebrospinal fluid formation and absorption in dehydrated sheep

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Chodobski, Adam, Joanna Szmydynger-Chodob ska, and Michael J. McKinley. Cerebrospinal fluid formation and absorption in dehydrated sheep. Am. J. Physiol. 275 (Renal Physiol. 44): F235–F238, 1998.—Cerebrospinal fluid (CSF) plays an important role in the brain's adaptive response to acute osmotic disturbances. In the present experiments, the effect of 48-h dehydration on CSF formation and absorption rates was studied in conscious adult sheep. Animals had cannulas chronically implanted into the lateral cerebral ventricles and cisterna magna to enable the ventriculocisternal perfusion. A 48-h water deprivation altered neither CSF production nor resistance to CSF absorption. However, in the water-depleted sheep, intraventricular pressure tended to be lower than that found under control conditions. This likely resulted from decreased extracellular fluid volume and a subsequent drop in central venous pressure occurring in dehydrated animals. In conclusion, our findings provide evidence for the maintenance of CSF production during mild dehydration, which may play a role in the regulation of fluid balance in the brain during chronic hyperosmotic stress.

Hypernatremia; vasopressin; ventriculocisternal perfusion; choroid plexus; organic osmolytes

CEREBROSPINAL FLUID (CSF) is predominantly produced by the choroid plexus (6). However, 10–30% of total CSF formation is extrachoroidal in origin and is represented by the bulk flow of interstitial fluid (4, 19). Under normal conditions, the interstitial fluid drains into the CSF space (4, 19). Acute hyperosmolality results in the reversal of the pressure gradient between the brain parenchyma and CSF (22), causing retrograde flow of CSF into the brain tissue (4, 19). Cserr and collaborators (18) have demonstrated that in acute hypernatremia the CSF that enters the brain is the major source of Na$^+$ and Cl$^-$ for the brain parenchyma. The brain's volume regulatory gain of Na$^+$ and Cl$^-$ facilitates the maintenance of the intracellular fluid space (at the expense of the extracellular compartment), as no change in intracellular water has been found as early as 30 min following the onset of hypernatremia (3). Thus CSF plays an integral role in the regulation of water and electrolyte balance in the brain during acute hyperosmotic stress.

During chronic hypernatremia, the brain tissue gradually accumulates organic osmolytes, and total brain water content slowly returns to normal levels (10). Since the transport systems for organic osmolytes, such as myo-inositol and amino acids, exist at the blood-CSF barrier (2, 12, 16, 17, 21), CSF may play a role in supplying these molecules to the brain parenchyma. It is not known, however, whether under chronic hyperosmotic conditions CSF formation is maintained, thereby allowing for effective distribution of organic osmolytes in the brain. Indeed, chronic hypernatremia promotes the release of vasopressin, a hormone which has been found to inhibit CSF formation (9). The aim of the present study was, therefore, to assess the production of CSF in sheep that had been dehydrated for 48 h. For completeness of analysis of CSF dynamics during water deprivation, CSF absorption was also evaluated.

MATERIALS AND METHODS

Animals. Four adult crossbred Merino ewes weighing between 37 and 46 kg were used. Several weeks before experimentation, the surgical procedures were performed under general anesthesia, which was induced with intravenous thiopentone sodium and maintained with halothane-O$_2$ inhalation mixture. Sheep were oophorectomized, and both carotid arteries were endothised into bilateral skin loops in the neck. The animals had three stainless steel guide tubes implanted in the brain. Two guide tubes (shaft length 20 mm) were positioned over each lateral ventricle (14), and the third guide tube (shaft length 31 mm) was introduced into the brain and directed toward the cisterna magna (1). The latter guide tube was implanted in the midline of the brain through a burr hole drilled in the occipital bone close to its junction with the parietal bone. The tip of this guide tube was positioned ~5 mm above the atlantoovalcical membrane. X-rays were used for guidance, and the guide tubes were fixed in place with stainless steel screws and dental acrylic. Access to the lateral ventricles and cisterna magna was gained with inner needles inserted through the respective guide tubes. The positions of the guide tubes were confirmed at autopsy.

Sheep were housed in individual metabolism cages at a room temperature of 20 ± 2°C. Water was provided ad libitum, and food (800 g oaten/lucerne chaff) was given once a day at 1630. Before the experiments began, sheep were acclimated to remaining in a sling that restricted their movement for several hours.

Measurement of CSF formation and absorption rates. CSF production was measured by the ventriculocisternal perfusion method, as previously described (1). Two control experiments (in normally hydrated animals) and one experiment following 48-h water deprivation were performed on each animal, in random order. At least 1 wk was allowed to elapse between experiments. On the day of the experiment (at ~0900), animals were restrained in a sling. A 19-gauge stainless steel needle of appropriate length was inserted into one of the lateral ventricles, and intraventricular pressure (IVP) was measured relative to the interaural level. Subsequently, another 19-gauge needle was introduced into the cisterna magna for perfusion of the ventriculocisternal system at a rate of 420 µl/min. Cerebral ventricles were perfused with artificial CSF (1) containing blue Dextran 2000 (1 mg/ml) as indicator. In dehydrated sheep, the composition of artificial CSF was modified, based on a separate analysis of CSF samples collected from 48-h dehydrated animals (see Table 1). IVP was continuously monitored throughout the course of the experiment via the T-connector inserted into the inflow line. The pressure drop associated with the resistance
Table 1. Changes in ionic composition and osmolality of CSF and plasma

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CSF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Na⁺]</td>
<td>153 ± 1</td>
<td>157 ± 1</td>
<td>159 ± 2</td>
</tr>
<tr>
<td>[K⁺]</td>
<td>2.96 ± 0.02</td>
<td>2.98 ± 0.04</td>
<td>3.08 ± 0.04</td>
</tr>
<tr>
<td>[Ca²⁺]</td>
<td>1.31 ± 0.01</td>
<td>1.33 ± 0.02</td>
<td>1.36 ± 0.02</td>
</tr>
<tr>
<td>[Mg²⁺]</td>
<td>0.91 ± 0.01</td>
<td>0.92 ± 0.01</td>
<td>0.95 ± 0.03</td>
</tr>
<tr>
<td>[Cl⁻]</td>
<td>132 ± 2</td>
<td>138 ± 1</td>
<td>139 ± 1</td>
</tr>
<tr>
<td>[HCO₃⁻]</td>
<td>32.6 ± 0.5</td>
<td>23.6 ± 0.5</td>
<td>23.6 ± 0.7</td>
</tr>
<tr>
<td>[H₂PO₄⁻ + HPO₄²⁻]</td>
<td>0.33 ± 0.03</td>
<td>0.33 ± 0.04</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>Osmolality, mosmol/kg</td>
<td>302 ± 1</td>
<td>311 ± 1</td>
<td>316 ± 3</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Na⁺]</td>
<td>147 ± 0</td>
<td>151 ± 0</td>
<td>154 ± 1</td>
</tr>
<tr>
<td>[K⁺]</td>
<td>4.68 ± 0.06</td>
<td>4.54 ± 0.04</td>
<td>4.58 ± 0.08</td>
</tr>
<tr>
<td>Osmolality, mosmol/kg</td>
<td>294 ± 1</td>
<td>302 ± 2</td>
<td>310 ± 2</td>
</tr>
</tbody>
</table>

*All values are in mmol/l (except osmolality) and are means ± SE obtained in five sheep. CSF, cerebrospinal fluid. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with control (Newman-Keuls test). *P < 0.05 and **P < 0.01 compared with 24-h dehydration (Newman-Keuls test).*

RESULTS

A 48-h dehydration resulted in a significant (P < 0.01) loss of body weight. Body weight dropped from a control value of 41 ± 2 to 38 ± 2 kg after water...
dehydration. This was accompanied by a significant (P < 0.01) increase in plasma protein concentration (7.7 ± 0.2 vs. 8.1 ± 0.2 g/dl in control vs. dehydration, respectively), which indicated that the extracellular fluid volume was diminished. Table 1 shows the changes in ion composition and osmolality of CSF and plasma following 24 and 48 h of water deprivation measured in a separate series of experiments (n = 5). At these two points there was a significant (P < 0.01 to 0.02) elevation of Na+ content (2–5%) and osmolality (3–6%) of both CSF and plasma, compared with the water-replete state. Dehydration also increased CSF Cl− and phosphates concentrations (Table 1).

During the course of the ventriculocisternal perfusion, sheep did not show any signs of pain or discomfort. The rate of CSF production in the normally hydrated animals (67 ± 3 µl/min) was comparable to that found in our previous experiments performed on conscious sheep (1). Dehydration did not affect CSF formation (67 ± 5 µl/min), nor did it significantly alter the resistance to CSF absorption (70 ± 4 vs. 60 ± 7 mmHg min ml−1 in the water-replete state vs. dehydration, respectively). Dehydration tended (P = 0.06) to decrease IVP that was measured before the commencement of the ventriculocisternal perfusion. IVP dropped from a control value of 3.1 ± 0.5 to 1.0 ± 0.6 mmHg after 48 h of water deprivation. Consequently, in dehydrated sheep there was a shift to the left in the linear relationship between IVP and Va (Fig. 1); however, the difference between the water-replete state and dehydration did not attain statistical significance.

Control MABP levels (87 ± 6 mmHg) did not differ from those found in dehydration (85 ± 6 mmHg). In comparison, 48-h water deprivation resulted in a significant (P < 0.01) drop in CVP (−4.4 ± 1.6 vs. −7.0 ± 1.6 mmHg in the water-replete state vs. dehydration, respectively).

**DISCUSSION**

To the best of our knowledge, this is the first study to evaluate CSF dynamics in dehydration. An important finding of this study is that CSF formation was not altered in sheep that were deprived of water for 48 h. Dehydration is accompanied by a number of adaptive hormonal responses that are aimed at conservation of body fluids, which include augmented vasopressin release into the blood stream. In the 48-h dehydrated sheep, plasma vasopressin concentration increased up to −11 pg/ml, which represents a 200% change above the control peptide levels (15). Vasopressin can substantially reduce blood flow to the choroid plexus and decrease CSF formation; however, these vasopressin actions require higher plasma hormone concentrations than those found in dehydrated sheep (8, 9). This conclusion is supported by the present findings.

IVP tended to decrease following water deprivation, which likely resulted from decreased extracellular fluid volume (15) and a subsequent fall in CVP occurring in dehydrated animals. Similar to CSF production, the resistance to CSF absorption was not changed after 48-h dehydration. The lack of changes in the resistance to CSF absorption was presumably associated with the parallel decrease in IVP and CVP.

CSF has been shown to play an important role in brain volume regulation during acute hyperosmotic disturbances (4). Under these conditions, initial loss of brain tissue water results in bulk flow of CSF into brain, which promotes an accumulation of inorganic osmolytes in the brain parenchyma (18). Consequently, the brain tissue water can be partially regained. In diabetes insipidus Brattleboro rats, in which basal CSF production is lower than that found in vasopressin-competent Long-Evans rats, brain tissue electrolyte uptake following hyperosmotic challenge is considerably impaired (7).

Chronic osmotic stress is associated with a slow accumulation of organic osmolytes in the brain parenchyma (10). Accumulation of organic osmolytes seems to be advantageous for brain cells because, unlike electrolytes, these solutes, when present at high concentrations, do not perturb cellular enzymes or alter protein structures (20). It is possible that during chronic hypernatremia, CSF provides the brain parenchyma with organic osmolytes, as CSF formation is maintained in dehydrated sheep. This CSF action would be analogous to that in acute hyperosmolality, when CSF supplies the brain tissue with Na+ and Cl− (18). To promote retrograde CSF flow into the brain tissue, the existence of a positive pressure gradient between CSF and interstitial fluid would be required. Such a pressure gradient has been shown to be maintained for only 1–2 h following hyperosmotic stress (22). However, the methodology used by these authors may have not allowed them to detect small changes in interstitial fluid pressure. Indeed, while under normal conditions there is a bulk flow of interstitial fluid from brain parenchyma into the CSF space (4, 19), a pressure gradient that would favor this fluid flow has not been demonstrated in the above study (22). Improved experimental methodology will be needed to find out whether a pressure gradient promoting retrograde CSF flow into brain exists during chronic hypernatremia.

Specific transporters for myo-inositol and amino acids, the osmolytes that accumulate in the brain tissue during chronic hyperosmolality, have been identified in the choroid plexus (2, 12, 16, 17, 21). Following the uptake at the blood-CSF barrier, these molecules could be distributed by CSF in the brain. The concentration of free myo-inositol in CSF is higher than that in plasma, and CSF is likely to act as a source of myo-inositol for the brain (6). Under normal conditions, the levels of most of the amino acids in CSF are lower than those found in plasma (6). The direction of the net flux of amino acids across the blood-CSF barrier depends on the amino acid and its concentrations in plasma and CSF (16). At present, it is not known, however, how the concentrations of amino acids and the direction of their movement through the blood-CSF barrier change during hyperosmotic stress. Future work will, therefore, be required to determine whether CSF plays a role of organic osmolyte supplier for the brain parenchyma in chronic hypernatremia.
REFERENCES


