Brain adaptation to acute hyponatremia in young rats

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Silver, Stephen M., Barbara M. Schroeder, Paul Bernstein, and Richard H. Sterns. Brain adaptation to acute hyponatremia in young rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1595–R1599, 1999.—Brain swelling after acute hyponatremia in prepubescent rats, in contrast to adults, has recently been associated with an increase in brain sodium and a high mortality that could be prevented by preadministration of testosterone. To reexamine the effect of acute hyponatremia in young brain, we measured brain water and solute content in prepubescent rats after induction of hyponatremia over 4 h with water and arginine vasopressin. An 18% decrease in plasma sodium was associated with a 13% increase in brain water and a decrease in brain sodium and glutamate contents. No animals died. To assess the effect of sex hormones on brain adaptation, prepubescent rats were pretreated with estrogen or testosterone before acute hyponatremia. Brain sodium and potassium contents were significantly reduced in comparison to normonatremia in testosterone-pretreated but not estrogen-pretreated animals. However, there was no difference between estrogen-pretreated and testosterone-pretreated groups in mortality or in brain contents of water, electrolytes, or major organic osmolytes. In conclusion, we found that brain adaptation to acute hyponatremia in prepubescent rats is similar to that observed in adults.

The paradoxical increase in brain sodium despite brain swelling gave support to the authors’ hypothesis that estrogens inhibit and testosterone stimulates brain Na-K-ATPase (4). However, their observations also imply a major effect of sex hormones on other brain solutes. The osmotic effect of a 30% decrease in plasma sodium concentration combined with an increase in brain sodium (and no offsetting decrease in potassium) should have massively increased brain water content, even more than the 30% increase that would be expected of a perfect osmometer. The fact that brain water increased by less than one-third of what would be expected implies that there was a substantial loss of other solutes that were not measured; organic osmolytes would be the most likely solutes because of their well-documented role in brain adaptation to osmotic stress. Thus these findings suggested to us that loss of brain organic osmolytes from cells might be altered by sex hormones.

In the present study, we assessed brain adaptations to acute hyponatremia in prepubescent rats by measuring brain water, electrolytes, and organic osmolytes. In addition, we assessed the effects of male and female hormones on this adaptive response.

METHODS

For these studies, Sprague-Dawley postpartum female rats with mixed (experiment I) or all female (experiment II) litters (Harlan) were received on postnatal day 5 and housed in an animal facility according to National Institutes of Health and institutional animal care and use guidelines.

Experiment I: Brain Adaptation to Acute Hyponatremia in Prepubescent Rats

Acute hyponatremia was induced in 12 10-day-old rats (6 males, 6 females) by subcutaneous injection of water (12% body wt, 3 subcutaneous injections at 0, 1, and 2 h) and arginine vasopressin (AVP) (5 mU sc at 0, 1, 2, and 3 h). Twelve sham-injected littersmates served as controls. Four hours after initial injection of water and AVP, animals were killed by decapitation. Trunk blood was obtained for measurement of plasma sodium. The dermis and skull were cut with operating scissors, and the skull cap was gently removed with microscope slide forceps. The brain was removed from the skull and cut in half. One hemisphere was immediately frozen in liquid nitrogen for measurement of organic osmolytes, and the other hemisphere was weighed 1 min after removal from the skull and saved for determination of tissue water and electrolyte content.

Results in control and hyponatremic groups, and males and females within each group, were compared by Student’s t-test (StatView II, Abacus Concepts, Berkeley, CA).

Experiment II: Effects of Testosterone vs. Estrogen on Brain Adaptation to Acute Hyponatremia in Prepubescent Female Rats

On postnatal day 7, female pups were divided into four experimental groups: 1) testosterone-treated control group, 2) testosterone-treated hyponatremia group, 3) estrogen-treated control group, and 4) estrogen-treated hyponatremia group. Animals were subcutaneously injected with either 10 mg/kg body wt testosterone enanthate (Sigma Chemical) or 0.33 µg/100 g body wt 17-ß-estradiol 3-benzoate (Sigma) in 100 µl vehicle (oil), doses that have previously been used in

Reports of fatal, acute postoperative water intoxica- tion in young women and children have generated interest in the effects of age, gender, and sex hormones on brain adaptations to hyponatremia (2, 3, 6).

Recently, in an intriguing set of experiments, Arrieff and co-workers (4) studied the effects of acute hyponatremia in prepubescent rats. A 30% decrease in plasma sodium over 4 h resulted in an 84% mortality and caused severe cerebral edema with an increase in brain sodium content, a finding opposite to the adaptive decrease in brain sodium that has been observed after acute hyponatremia in adult animals. Remarkably, both mortality and the increase in brain sodium after acute hyponatremia in prepubescent animals were completely prevented by pretreatment with testosterone.

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Hyponatremia in Prepubescent Rats

Experiment I: Brain Adaptation to Acute Hyponatremia in Young Rats

Results in the four experimental groups were compared by ANOVA (Scheffé’s F test).

Analytical Procedures

Plasma sodium. Plasma sodium was measured by flame photometry (Instrumentation Laboratory model 943, Boston, MA).

Brain water and electrolytes. A hemisphere of brain was dried at 100°C for 48 h and reweighed to determine water content. The dried tissue was then crushed and dissolved in 0.75 M HNO₃ for sodium and potassium analysis by flame photometry.

Brain organic osmolyte content. The method for measurement of organic osmolyte content in brain has been described previously (19). Frozen brain tissue was crushed under liquid nitrogen and lyophilized. Amino acid content of lyophilized brain tissue was then determined by HPLC after derivitization with phenyl isothiocyanate (Pico Tag, Waters, Milford, MA). Methionine sulfone served as the internal standard. The contents of myo-inositol and creatine were determined by HPLC, utilizing a Sugar Pak II column (Waters). Maltose served as the internal standard. Levels of organic osmolytes were quantified on a Waters 840 chromatography control station in experiment I and a Waters Millenium 2001 chromatography workstation in experiment II.

RESULTS

Experiment I: Brain Adaptation to Acute Hyponatremia in Prepubescent Rats

All data are presented as means ± SE. There were no significant differences between male and female animals in plasma sodium concentration or in brain water, electrolyte, and major organic osmolyte contents (Table 1). Brain water increased by 13% despite an 18% decrease in plasma sodium concentration, indicating a degree of brain adaptation to the acute decrease in plasma tonicity. Indeed, brain sodium content after hyponatremia was significantly lower in hyponatremic animals; brain potassium content was not significantly decreased. The sum of five major organic osmolytes did not change after hyponatremia (Table 1). When compared individually, brain content of glutamate decreased significantly after hyponatremia but that of other organic osmolytes did not change (Fig. 1). Brain content of phosphoethanolamine was more than twofold higher (14.6 ± 0.5 in control and 13.7 ± 0.4 mmol/kg dry tissue wt in hyponatremic animals) in prepubescent animals compared with what was previously reported in adult rats (6.3 ± 0.3 mmol/kg dry tissue wt) (20). Higher levels of brain phosphoethanolamine in young animals have been attributed to the compound’s role as a precursor of membrane phospholipid and myelin (18).

Experiment II: Effects of Testosterone vs. Estrogen on Brain Adaptation to Acute Hyponatremia in Prepubescent Female Rats

Values for plasma sodium and for brain water and electrolyte content are given in Table 2. Plasma sodium concentrations in control and hyponatremic groups were equivalent in testosterone- and estrogen-pre-treated animals. There were no significant differences
in brain water or electrolyte contents between testosterone- and estrogen-pretreated animals in either control or acutely hyponatremic groups. Baseline brain water and sodium contents in testosterone-treated animals tended to be higher than in animals pretreated with estrogen, but these changes were not statistically significant. After induction of acute hyponatremia, brain sodium and potassium contents were significantly reduced in comparison to normonatremia, but only in animals pretreated with testosterone. As in experiment 1, brain glutamate content was significantly decreased after hyponatremia in both testosterone- and estrogen-treated animals but no other significant changes in brain content of summed (Table 2) or individual (Fig. 2) major organic osmolytes were observed.

**DISCUSSION**

We find that in prepubescent rats acute hyponatremia is associated with an adaptive decrease in brain sodium content which militates against osmotic brain swelling, a pattern of brain adaptation like that of adults. Brain adaptation to hypernatremia in adult and prepubescent rats has also been found to be similar (23). In our studies, brain content of glutamate decreased after acute hyponatremia, but no other changes in brain content of major organic osmolytes were observed. This is consistent with the minor role that organic osmolytes play in the acute phase of adaptation to changes in osmolality (11). As in most studies of acute hyponatremia in adult animals, mortality in prepubescent animals was low (1, 22). Mortality was also unaffected in our studies by pretreatment with testosterone or estrogen. Brain water, sodium, potassium, and organic osmolyte contents were virtually identical in hyponatremic prepubescent females pretreated with testosterone or estrogen. However, significant adaptive loss of electrolytes from brain after acute hyponatremia did not occur in animals pretreated with estrogen.

In adult animals, loss of brain sodium occurs within 1 h after induction of acute hyponatremia and mitigates cerebral edema (15). The loss of brain sodium is presumed to result from increased intracranial pressure, which drives bulk flow of sodium-rich fluid from the brain’s extracellular space to the cerebrospinal fluid and then to the circulation. In contrast, acute hyponatremia in 3-day-old dogs increases brain water in proportion to the decrease in plasma sodium, without a decrease in brain sodium content (17). Thus, without the adaptive loss of sodium, the brain behaved like a perfect osmometer. The authors of that study suggested that the pliable cranium of young animals prevented the increase in intracranial pressure required to induce bulk flow of interstitial fluid. Our finding that brain sodium contents decrease in acutely hyponatremic prepubescent rats implies that the cranial vault of young rats is less compliant than that of the newborn dog. However, the effect of skull compliance on the adaptation to hyponatremia is uncertain; disruption of the cranial vault in adult animals before induction of

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<th>Testosterone-Pretreated Control (n=15)</th>
<th>Estrogen-Pretreated Control (n=14)</th>
<th>Testosterone-Pretreated Hyponatremic (n=15)</th>
<th>Estrogen-Pretreated Hyponatremic (n=14)</th>
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<tbody>
<tr>
<td>Plasma sodium, mM</td>
<td>140±2</td>
<td>141±2</td>
<td>118±2*</td>
<td>119±2*</td>
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<td>Brain water, l/kg dry tissue wt</td>
<td>6.15±0.05</td>
<td>5.98±0.05</td>
<td>6.73±0.07*</td>
<td>6.59±0.06*</td>
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<tr>
<td>Brain sodium, mmol/kg dry tissue wt</td>
<td>386±6</td>
<td>365±6</td>
<td>353±8†</td>
<td>353±7†</td>
</tr>
<tr>
<td>Brain potassium, mmol/kg dry tissue wt</td>
<td>649±3</td>
<td>638±5</td>
<td>626±4†</td>
<td>631±7†</td>
</tr>
<tr>
<td>Major brain organic osmolyte content, mmol/kg dry tissue wt</td>
<td>182±4</td>
<td>189±4</td>
<td>178±3</td>
<td>180±8</td>
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Values are means ± SE. Major organic osmolyte content is sum of taurine, creatine, myo-inositol, glutamate, and glutamine contents. *P < 0.05 vs. both pretreatment groups; †P < 0.05 vs. testosterone-pretreated control.
Hyponatremia has not been shown to prevent a decrease in brain sodium content or an increase in cerebrospinal fluid pressure (12, 14). Our findings in prepubescent animals differ significantly from those of the similar study of Arieff and co-workers (4). These investigators reported a high mortality and an increase in brain sodium in 11-day-old rats after induction of acute hyponatremia. Because the survival rate in their studies was so poor, measures of brain constituents were apparently performed on only 6 of the 44 animals studied. Thus the finding of an increased brain sodium content after acute hyponatremia in this group might reflect a premorbid state in these animals. In contrast, all animals in our study survived. In the experiments of Arieff et al. (4), hyponatremia was induced by a single bolus of intraperitoneal glucose in water. Intraperitoneal injection of isosmotic glucose has been associated with extracellular volume depletion and hypotension in experimental animals (7, 10). We gave several subcutaneous injections of water over 2 h to induce hyponatremia more gradually. These differences in methodology may explain the improved survival in our studies.

After induction of hyponatremia, the animals in the study by Arieff et al. (4) had more severe hyponatremia than in our study (98 mM vs. 113 mM). However, their 11-day-old animals were also more hyponatremic at baseline (124 mM) than our 10-day-old controls (135 mM) or our 12-day-old animals pretreated with estrogen or testosterone (140 mM). Our values are also slightly higher than those of Trachtman and co-workers (23), who found a plasma sodium of 133 mM in 12-day-old animals. The 22 mM decrease in serum sodium concentration in our study after induction of acute hyponatremia was comparable to the 26 mM decrement obtained by Arieff et al. (4). As previously noted, Arieff et al. found that pretreatment with testosterone increased survival after acute hyponatremia from 16% to 100%. However, hyponatremia in the testosterone-pretreated group appeared less severe than in animals that received no testosterone pretreatment (106 ± 1 vs. 98 ± 2 mM); it was not specifically stated whether these values were compared statistically.

Arieff and co-workers (4) proposed that low levels of brain Na-K-ATPase activity in young rats prevent extrusion of sodium from brain cells, making the young rats less able to adapt to hyponatremia than adult animals. Noting that estrogen decreases and testosterone increases Na-K-ATPase in cultured astrocytes (9), Arieff et al. (4) suggested that pretreatment with testosterone in young animals increased brain Na-K-ATPase activity, enhancing sodium loss from brain cells after acute hyponatremia and improving survival. Our finding that brain sodium content fell when hyponatremia was induced in testosterone-treated animals but not in those treated with estrogen is consistent with the hypothesis proposed by Arieff and co-workers (4). However, brain potassium content also decreased in testosterone-treated animals. Enhancement of Na-K-ATPase activity alone would be expected to promote potassium retention as well as loss of sodium. Other ion channels that transport both sodium and potassium from cells could potentially mediate hormonal effects on the brain's adaptation to hyponatremia (13).

Significant loss of brain sodium after hyponatremia was observed in normal and testosterone-pretreated animals but not in those pretreated with estrogen, implying that estrogen limits adaptive loss of brain sodium in acute hyponatremia. The importance of this finding is unclear, since no significant differences in brain water or electrolytes at baseline or after hyponatremia were found between animals treated with male or female sex hormones. In addition, no differences in brain water or electrolyte content after hyponatremia have been found between adult male and female animals. Fraser and co-workers (8) found a higher mortality in adult female rats, but these investigators did not measure brain solute or water contents. Arieff and colleagues (4) observed no differences in brain water or electrolyte contents when comparing hyponatremic male and female animals treated with AVP, and, in contrast to the findings of Arieff et al. (4) and Fraser et al. (8), mortality was low for both sexes.

In conclusion, we found brain adaptation to acute hyponatremia in prepubescent rats similar to that observed in adult animals. Exogenous estrogen may have limited the adaptive loss of electrolytes from the brain after acute hyponatremia in young animals, but the mechanism and significance of this effect are unclear.

Perspectives

There has been substantial controversy over the claim that children and females are at an increased risk for brain injury from hyponatremia (3, 5, 25). The recent study in young rats by Arieff and co-workers (4) yielded two remarkable findings that supported this contention: 1) in young animals, acute hyponatremia caused an increase in brain sodium, a response qualitatively different from that observed in adults, and 2) testosterone pretreatment reversed this response and dramatically improved survival. In the present study, these findings were not confirmed; brain adaptation to acute hyponatremia in young animals was qualitatively similar to that in adults, and pretreatment with testosterone or estrogen did not alter survival. However, there have been relatively few studies in animals or in cell lines that investigated the ontogeny of brain adaptation to osmotic perturbation; such studies would be valuable and would help to resolve the discrepancy between the conclusions of Arieff et al. (4) and our own conclusions. Large, prospective human studies will be necessary to resolve whether women and children are indeed more susceptible to brain injury from hyponatremia. Until then, prevention of rapid changes in plasma...
tonicity should be pursued with equal vigilance in all patients.

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