Rapid (24-Hour) Reaccumulation of Brain Organic Osmolytes (Particularly myo-Inositol) in Azotemic Rats after Correction of Chronic Hyponatremia

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Abstract. It was recently demonstrated that renal failure and exogenous urea prevent myelinolysis induced by rapid correction of experimental hyponatremia. To determine why elevated blood urea levels favorably affect brain tolerance to osmotic stress, the changes in brain solute composition that occur when chronic hyponatremia is rapidly corrected were studied in rats with or without mercuric chloride-induced renal failure. After 48 h of hyponatremia, the brains of azotemic and nonazotemic animals became depleted of sodium, potassium, and organic osmolytes. Twenty-four hours after rapid correction of hyponatremia, the brains of animals without azotemia remained depleted of organic osmolytes, with little increase in myo-inositol or taurine contents above those observed in animals with uncorrected hyponatremia; brain electrolytes were rapidly reaccumulated, increasing the brain sodium content to a level 17% higher than values for normonatremic control animals. In contrast, within 2 h after correction of hyponatremia, brain myo-inositol contents in azotemic rats returned to control levels and brain taurine levels were significantly higher than those in azotemic animals with uncorrected hyponatremia (16.5 versus 9 µmol/g dry weight). There was no “overshooting” of brain sodium and water contents after rapid correction in the azotemic animals. Rapid reaccumulation of brain organic osmolytes after correction of hyponatremia could explain why azotemia protects against myelinolysis.

Organic osmolytes play a key role in cell volume homeostasis. Several organs, most notably the kidney and brain, are particularly dependent on adaptive mechanisms that regulate their volume during osmotic perturbations (1–3). The brain adapts to chronic (1- to 2-d) hyponatremia by extruding electrolytes and organic osmolytes, thus limiting brain cell swelling. Conversely, during correction of chronic hyponatremia, the restoration of brain osmolyte contents counters brain dehydration; however, this process normally requires several days for completion (4,5). Depletion of brain osmolytes during adaptation to chronic hyponatremia makes the brain vulnerable to injury when the hyponatremia is corrected. Brain myelinolysis is a well recognized consequence of excessive correction of chronic hyponatremia (6–8). Among human subjects, additional risk factors, such as hypokalemia, chronic liver disease, diuretics (thiazides), or malnutrition, could also be implicated in the pathogenesis of this demyelinating disease (6). Areas of more severe injury are topographically correlated with sites that are more depleted of organic osmolytes after correction of hyponatremia (9).

Urea has been successfully used to treat hyponatremia (10–12). In previous studies, we demonstrated that exogenous urea protected animals against myelinolysis in a rat model of hyponatremia (6,13,14). Similarly, clinical observations suggest that uremic patients can tolerate large fluctuations in serum sodium levels without developing myelinolysis (15). We recently confirmed this hypothesis in our experimental model, demonstrating that azotemic (48 h) rats with hyponatremia tolerated large corrections in serum sodium levels without brain damage (16).

To determine why elevated blood urea levels favorably affect the tolerance of the brain to osmotic stress, we studied the changes in brain solute composition that occur when chronic hyponatremia is rapidly corrected in rats with or without azotemia. The results demonstrate that an azotemic environment enhances the recovery of organic osmolytes by the brain and prevents the “overshoot” of brain sodium levels that occurs when chronic hyponatremia is rapidly corrected in nonazotemic animals.

Materials and Methods

Animals

Male Wistar rats, weighing 250 to 300 g, were used in these experiments. The animals were housed in individual cages for at least 3 d before the beginning of the study. The animals were maintained on
a standard diet of pelleted rat chow and were given free access to water. The mean room temperature was controlled at 25°C, with lights on from 7:00 a.m. to 7:00 p.m.

**Induction of Hyponatremia (Days 1 to 3)**

Chronic (3-d) severe hyponatremia (serum sodium concentration, <120 mEq/L) was induced as described previously (16), with continuous infusion of arginine-8-vasopressin, via subcutaneous osmotic pumps (model 2001; Alzet, Palo Alto, CA), at a rate of 24 mU/h and with intraperitoneal injections of 2.5% (140 mM) d-glucose in water. The mean room temperature was controlled at 25°C. The supernatant was subsequently deproteinized with a 10,000 Mw cutoff ultrafiltration unit (Millipore), dried under nitrogen, and stored desiccated at 15°C.

**Correction of Hyponatremia (Day 4)**

Hyponatremia was corrected on day 4, with intraperitoneal injections of hypertonic saline solution. Rats received one injection (at 12:00 noon) of 1 M NaCl at a dose of 2.5 ml/100 g body wt. This dose was previously demonstrated to increase serum sodium levels by >25 mEq/L per 24 h and to cause a high incidence of myelinolysis. Arginine-8-vasopressin infusion was maintained throughout all experiments (16).

**Blood Measurements**

Blood samples (0.25 ml) were collected via tail transection, with light ethyl ether anesthesia, for serum sodium, urea, and creatinine level measurements (ABL 500; Radiometer, Copenhagen, Denmark).

**Brain Composition**

At the end of the experimental procedures, rats were decapitated and brains were rapidly removed and weighed. Each brain was subsequently bisected, and one hemisphere was weighed for calculation of brain water contents. Hemispheres were frozen and stored at −70°C for determination of tissue organic osmolyte, water, and electrolyte contents.

**Analytical Procedures**

**Brain Water and Electrolyte Contents.** One hemisphere of each brain was dried at 100°C for 48 h and reweighed for determination of brain water contents. The dried tissue was then reweighed and extracted for 48 h with 0.75 N hydrochloric acid, for analysis of sodium and potassium levels by flame photometry (IL943; Instrumentation Laboratories, Lexington, MA).

**Brain Organic Osmolyte Contents.** myo-Inositol, urea, betaine, glycerophosphorylcholine, and creatine levels were measured with modifications of the methods described by Wolff et al. (17). Frozen brain hemispheres were crushed in liquid nitrogen, lyophilized, and stored desiccated at −70°C until use. At the time of analysis, a 10- to 15-mg fraction of each specimen was weighed and extracted with 500 μl of ice-cold 6% perchloric acid containing 1 mM malate (as an internal standard). Samples were centrifuged in a Beckman TJ-6 centrifuge (Beckman Instruments, Inc., Fullerton, CA) at 1500 × g for 10 min at 4°C. The resulting supernatant was neutralized with the addition of 3 N KOH/50 mM K₂HPO₄. The salt complex was removed by centrifugation as described above, and the neutralized supernatant was transferred to a 16- × 100-mm polypropylene tube, flash-frozen in liquid nitrogen, and lyophilized. The lyophilized brain tissue was resuspended in 250 μl of HPLC-grade water and filtered through a type HV Millex membrane (0.45 μm; Millipore Corp., Milford, MA), into a limited vial insert. A 50-μl aliquot was injected into a Waters HPLC system (Waters Corp., Milford, MA), fractionated on a SugarPak 1 column (Waters), and eluted with 0.1 mM calcium disodium ethylenediaminetetraacetate at 0.55 ml/min at 80°C. Metabolites were detected with a model 410 differential refractometer (Waters) and quantified at a Waters Millennium 2001 chromatography workstation.

**Amino Acids.** Amino acids were determined with a modification of the method described by Gunawan et al. (18). Briefly, 10 to 15 mg of brain lyophilized brain tissue were weighed and extracted with 400 μl of ice-cold 0.1 N hydrochloric acid containing 0.4 mM methionine sulfone (as an internal standard). The samples were centrifuged as described above. The acid supernatant was clarified by centrifugation in a benchtop Sorvall centrifuge (DuPont, Wilmington, DE) for 2 min at 4°C. The supernatant was subsequently deproteinized with a 10,000 MWDL Ultrafree-MC centrifugal filter unit (Millipore), dried under vacuum, and derivatized with phenylisothiocyanate. Fifty-microliter sample aliquots were injected into a Waters HPLC system, and amino acids were separated on a PicoTag column (Waters) and measured with a model 441 absorbance detector. Results were quantified as described previously.

**Statistical Analyses**

Data are generally presented as mean ± SEM values. Comparisons between groups were assessed with one-factor ANOVA, and significance was determined with the Scheffé F test (Statview 512⁺; Brain Power, Calabasas, CA). Significance was accepted at P < 0.05.

**Experimental Groups (Groups I to IX)**

**Group I: Control (n = 10).** Ten normal normotensive rats were decapitated for measurement of serum sodium, urea, and creatinine levels and determination of brain organic osmolyte composition.

**Group II: Normonatremia after Exogenous Urea (n = 10).** In 10 normotensive rats, brain composition was assessed 8 h after the administration of exogenous urea. Rats in this group were given one subcutaneous injection of HgCl₂ at 7:00 a.m. Urea (24%) was injected 2 h later (1.5 ml/100 g body wt, administered intraperitoneally). Animals were decapitated at 5:00 p.m. for brain analysis and serum sodium, urea, and creatinine concentration determinations.

**Group III: Normonatremia with Azotemia (48 h) (n = 16).** In 16 normotensive rats, brain composition and serum parameters were determined 48 h after subcutaneous injection of HgCl₂. Caloric supplements were provided by oral administration of 5% d-glucose in water (5% body wt, in two fractionated doses on days 1 and 3), equivalent to the amount used in the hyponatremic model.

**Group IV: Hyponatremia (3 d) (n = 16).** In 16 hyponatremic rats, brain composition and serum parameters were determined 48 h after subcutaneous injection of HgCl₂. Caloric supplements were provided by oral administration of 5% d-glucose in water (5% body wt, in two fractionated doses on days 1 and 3), equivalent to the amount used in the hyponatremic model.

**Group V: Hyponatremia (3 d) and Azotemia (48 h) (n = 14).** In 14 rats, hyponatremia was induced for 3 d as described previously. Azotemia was induced by the subcutaneous administration of HgCl₂ on day 2. Brain and blood analyses were performed on day 4 (48 h after injection of HgCl₂), without correction of serum sodium levels.

**Group VI: Hyponatremia (3 d) 2 h after Correction with NaCl (n = 14).** In 14 rats, hyponatremia was induced as described previously. Serum sodium levels were then corrected on day 4 with...
intraperitoneal injection of 1 M NaCl at a dose of 2.5 ml/100 g body wt. Brain and blood analyses were performed 2 h after the NaCl injection.

Group VII: Hyponatremia (3 d) 24 h after Correction with NaCl (n = 17). In 17 rats, hyponatremia was induced as described previously and serum sodium levels were corrected on day 4, as for group VI. In this group, however, the brain and blood analyses were performed 24 h after NaCl administration.

Group VIII: Hyponatremia (3 d) plus Azotemia (48 h) 2 h after Correction with NaCl (n = 18). In 18 rats, hyponatremia was induced as described previously. HgCl₂ was administered subcutaneously on day 2. Brain and blood analyses were performed on day 4, 2 h after the intraperitoneal NaCl injection and 50 h after the HgCl₂ injection.

Group IX: Hyponatremia (3 d) plus Azotemia (48 h) 24 h after Correction with NaCl (n = 19). In 19 rats, hyponatremia and azotemia were induced as for group VIII. Serum sodium levels were corrected on day 4. Brain and blood analyses were performed 24 h after NaCl administration in this group.

Results

Blood Measurements

Serum sodium and urea values are presented in Table 1. All animals treated with vasopressin and intraperitoneally administered d-glucose and water developed severe hyponatremia (serum sodium concentration, <120 mEq/L), with no significant difference between groups.

Animals treated with HgCl₂ developed significant renal failure, with serum urea levels markedly higher than those observed without HgCl₂ injection. The mean serum creatinine value for the azotemic group after 3 d of hyponatremia (group V) was 4.3 ± 0.5 mg/dl. The level of azotemia was generally well tolerated by the rats, with only moderate decreases in spontaneous activity and occasionally increased irritability. Nonhyponatremic animals given HgCl₂ (group III) developed less severe azotemia than did groups with hyponatremia, i.e., 293 mg/dl versus 406 mg/dl (group VIII) or 418 mg/dl (group IX) (P < 0.001), but exhibited blood urea levels similar to those in normonatremic rats given exogenous urea (group II) (293 versus 272 mg/dl; NS). In group IX, serum urea levels increased significantly between day 4 (before correction, 418 mg/dl) and day 5 (24 h after correction, 544 mg/dl) (P < 0.001) (16).

In the hyponatremic groups, administration of hypertonic saline solution increased serum sodium concentrations by ≥25 mEq/L after the first 24 h of correction in all animals. Twenty-four hours after hypertonic saline solution injection, at the time of decapitation, most (14 of 18 animals, 78%) of the nonazotemic rats (group VII) had developed typical neurologic symptoms of brain myelinolysis (13,16), including gait ataxia and marked neuromuscular hyperexcitability. Four animals seemed to be moribund. In contrast, only one of the 19 azotemic rats (5%) that underwent correction with hypertonic saline solution (group IX) exhibited any neurologic abnormalities, and these were minor (P < 0.001). The serum urea level in the single animal with neurologic abnormalities was 544 mg/dl.

### Table 1. Serum sodium levels, urea levels, and brain weights in the different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Sodium (mEq/L)</th>
<th>Urea Level (mg/dl)</th>
<th>Brain Weight (g)</th>
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<tbody>
<tr>
<td>Group I</td>
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<tr>
<td>(n = 17)</td>
<td>138 ± 3</td>
<td>272 ± 42</td>
<td>1.92 ± 0.13</td>
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<tr>
<td>Group II</td>
<td>138 ± 3</td>
<td>272 ± 42</td>
<td>1.94 ± 0.11</td>
</tr>
<tr>
<td>(n = 17)</td>
<td>142 ± 3</td>
<td>239 ± 71</td>
<td>1.91 ± 0.08</td>
</tr>
<tr>
<td>Group III</td>
<td>140 ± 4</td>
<td>239 ± 71</td>
<td>1.91 ± 0.08</td>
</tr>
<tr>
<td>Group IV</td>
<td>100 ± 5</td>
<td>243 ± 9</td>
<td>1.84 ± 0.06</td>
</tr>
<tr>
<td>(n = 17)</td>
<td>109 ± 6</td>
<td>243 ± 9</td>
<td>1.84 ± 0.06</td>
</tr>
<tr>
<td>Group V</td>
<td>100 ± 5</td>
<td>243 ± 9</td>
<td>1.84 ± 0.06</td>
</tr>
<tr>
<td>Group VI</td>
<td>103 ± 7</td>
<td>579 ± 17</td>
<td>1.75 ± 0.10</td>
</tr>
<tr>
<td>(n = 17)</td>
<td>103 ± 7</td>
<td>579 ± 17</td>
<td>1.75 ± 0.10</td>
</tr>
<tr>
<td>Group VII</td>
<td>105 ± 7</td>
<td>579 ± 17</td>
<td>1.75 ± 0.10</td>
</tr>
<tr>
<td>Group VIII</td>
<td>105 ± 7</td>
<td>579 ± 17</td>
<td>1.75 ± 0.10</td>
</tr>
<tr>
<td>Group IX</td>
<td>105 ± 7</td>
<td>579 ± 17</td>
<td>1.75 ± 0.10</td>
</tr>
</tbody>
</table>

* SNa, serum sodium level; ΔSNa, gradient of correction (2 or 24 h) after NaCl. Values are mean ± SD. Group I, controls; Group II, normonatremic rats 8 h after exogenous urea; Group III, hyponatremia (3 d); Group IV, hyponatremia and azotemia; Group V, hyponatremia and azotemia 24 h after NaCl; Group VII, hyponatremia and azotemia 24 h after correction with NaCl.
Brain Composition

Brain Water and Electrolyte Contents. In both azotemic and nonazotemic rats, administration of NaCl was followed by an initial decrease in brain weight. Brain weights had decreased 9% in nonazotemic rats ($P < 0.001$) and 7% in azotemic rats ($P < 0.001$) at 2 h after correction. Twenty-four hours after correction, brain weights were almost completely normalized in both azotemic and nonazotemic rats, with no significant difference between the groups (Table 1).

Table 2 summarizes the effects of rapid correction of hyponatremia on total brain water, sodium, and potassium contents. Basal compositions for control animals, normonatremic rats with 48 h of azotemia (group III), and normonatremic rats with 8 h of azotemia after injection of exogenous urea (group II) were similar. In particular, there were no decreases in brain water contents in the azotemic groups. After 3 d of hyponatremia, brain water contents were significantly higher than control values, without significant differences between nonazotemic (group IV, +5% versus group I) and azotemic (group V, +2% versus group I) groups (6). Brain cation contents (sodium plus potassium) were similarly decreased in groups IV and V.

Two hours after correction of hyponatremia, brain water contents were significantly decreased in both group VI (−7.7% versus group IV and −2.8% versus group I) and group VIII (−7.6% versus group V and −5.8% versus group I). Brain water contents for nonazotemic (group VI) and azotemic (group VIII) rats were similar at that time.

Twenty-four hours after correction of hyponatremia, brain water contents were increased in the nonazotemic rats (group VII, +7% versus group I), whereas brain water contents had returned to levels that did not differ significantly from control values in the azotemic rats (group IX). Brain water contents for nonazotemic rats (group VII) were significantly higher than those for azotemic rats (group IX) ($P < 0.0002$).

After correction of hyponatremia, brain electrolytes (sodium plus potassium) were rapidly reaccumulated in both nonazotemic and azotemic groups. However, 24 h after correction of hyponatremia, brain sodium contents were significantly higher in nonazotemic rats, compared with azotemic rats (group VII versus group IX, $P < 0.0001$). At 24 h, brain potassium levels were comparable to control levels in both azotemic and nonazotemic groups (4,5).

Brain Organic Osmolyte Contents. Table 3 and Figures 1 and 2 demonstrate the changes in the most quantitatively important organic osmolytes (those with brain contents of >5 μmol/g dry weight) during chronic (3-d) hyponatremia and its subsequent correction in the nine groups. Table 4 presents organic osmolytes present at lower concentrations in the same groups.

The brain contents of the most quantitatively important organic osmolytes (myo-inositol, glutamate, glutamine, taurine, urea, γ-aminobutyric acid, aspartate, and phosphoethanolamine) were not statistically different in normonatremic control animals with or without azotemia (Table 3). As expected, brain urea contents were significantly higher in azotemic rats (groups II and III) than in control animals. Therefore, 48 h of

### Table 2. Brain water and electrolyte contents

<table>
<thead>
<tr>
<th>Group</th>
<th>Sodium content (Eq/g dry brain weight)</th>
<th>Potassium content (Eq/g dry brain weight)</th>
<th>Urea content (liters H2O/kg dry brain weight)</th>
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<tbody>
<tr>
<td>I</td>
<td>3.46 ± 0.02</td>
<td>3.03 ± 0.02</td>
<td>1.8 ± 0.02</td>
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<tr>
<td>II</td>
<td>3.48 ± 0.02</td>
<td>2.87 ± 0.02</td>
<td>2.1 ± 0.02</td>
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<tr>
<td>III</td>
<td>3.48 ± 0.02</td>
<td>3.03 ± 0.02</td>
<td>2.1 ± 0.02</td>
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<tr>
<td>IV</td>
<td>3.43 ± 0.02</td>
<td>2.78 ± 0.02</td>
<td>1.9 ± 0.02</td>
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<tr>
<td>V</td>
<td>3.40 ± 0.02</td>
<td>2.59 ± 0.02</td>
<td>1.7 ± 0.02</td>
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<tr>
<td>VI</td>
<td>3.41 ± 0.02</td>
<td>2.87 ± 0.02</td>
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<td>VII</td>
<td>3.39 ± 0.02</td>
<td>2.64 ± 0.02</td>
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<tr>
<td>VIII</td>
<td>3.37 ± 0.02</td>
<td>2.62 ± 0.02</td>
<td>1.7 ± 0.02</td>
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<tr>
<td>IX</td>
<td>3.36 ± 0.02</td>
<td>2.61 ± 0.02</td>
<td>1.7 ± 0.02</td>
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Table 3. Major brain organic osmolyte contents

<table>
<thead>
<tr>
<th>Compound</th>
<th>Group I (0.05)</th>
<th>Group II (0.06)</th>
<th>Group III (0.06)</th>
<th>Group IV (0.06)</th>
<th>Group V (0.05)</th>
<th>Group VI (0.04)</th>
<th>Group VII (0.03)</th>
<th>Group VIII (0.01)</th>
<th>Group IX (0.01)</th>
<th>Group X (0.01)</th>
<th>Group XI (0.01)</th>
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<tr>
<td>Glutamate</td>
<td>9.00 ± 0.38</td>
<td>11.59 ± 0.46</td>
<td>12.55 ± 0.38</td>
<td>12.55 ± 0.46</td>
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<td>Glutamine</td>
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<td>Creatine</td>
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<td>Myo-inositol</td>
<td>1.90 ± 0.10</td>
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</table>

*Table 3. Major brain organic osmolyte contents.

Note: Data are mean ± SEM.

Osmolytes in Azotemia and Hyponatremia

azotemia by itself did not increase brain organic osmolyte contents.

With the exception of brain urea contents, the brain contents of the major organic osmolytes were reduced to similar degrees by hyponatremia in nonazotemic (group IV, −75.8 μmol/g dry weight, −44%) and azotemic (group V, −6 μmol/g dry weight, −33%) groups. Only brain taurine contents were significantly higher in group V (group V versus group IV, P < 0.05). Basal levels of taurine in the normonatremic azotemic rats (group III) were not different from control values, however.

After rapid correction of hyponatremia, brain myo-inositol contents in the azotemic groups returned to normal levels within 2 h and remained stable at 24 h after correction. In hyponatremic rats without azotemia, the brain contents of myo-inositol remained 5% lower than control values even 24 h after correction, i.e., significantly lower than postcorrection values for the azotemic groups (group VI versus group VIII and group VII versus group IX, P < 0.0001).

Similarly, brain glutamine and taurine levels were normalized 24 h after correction in azotemic animals but the levels of these brain amino acids remained low in nonazotemic animals. Within 2 h after correction, brain taurine contents had increased by 32%, whereas no change was observed in nonazotemic rats. Creatine and aspartate levels also increased significantly more rapidly in azotemic rats than in nonazotemic rats. In both azotemic and nonazotemic groups, brain glutamate concentrations increased rapidly (2 h) after correction and levels returned to the normal range by 24 h.

Among the “minor” brain amino acids (contents of <5 μmol/g dry weight), no significant differences were noted between the groups except for threonine, tyrosine, methionine, phenylalanine, and ornithine, which were present at higher concentrations 24 h after correction in azotemic rats (Table 4). Alanine and lysine levels were normalized in group IX but were significantly lower than group VII levels.

By 2 h after correction, the total contents of the major brain organic osmolytes had increased to 95% of the control value in the azotemic group (group VIII, 162 μmol/g dry weight; group I, 170 μmol/g dry weight) (Table 3). The total contents of the major brain organic osmolytes remained at 68% of control values in the nonazotemic group (group VI, 116 μmol/g dry weight; P < 0.0001). After 24 h, the level of brain organic osmolytes in nonazotemic rats was 75% of the control value (group VII, 128 μmol/g dry weight). In the azotemic group, the sum of the major brain organic osmolytes was 189 μmol/g dry weight (+11% versus control value, 170 μmol/g dry weight). The absolute difference in the brain contents of 10 major organic osmolytes (not including urea) between groups at 24 h after correction was 61 μmol/g dry weight (group VII, 128 μmol/g dry weight; group IX, 189 μmol/g dry weight). The difference in the total amounts of organic osmolytes (n = 25) between nonazotemic and azotemic rats at 24 h was also 61 μmol/g dry weight (group VII, 151 μmol/g dry weight; group IX, 212 μmol/g dry weight). Expressed as a function of brain tissue water (rather than dry tissue weight), the organic os-
molyte (excluding urea) concentration difference between azotemic and nonazotemic animals equaled 17 mM.

**Discussion**

Hyponatremic rats with azotemia tolerate rapid large increases in serum sodium concentrations without developing brain damage (16). Hyponatremic animals without azotemia that are subjected to the same osmotic stress exhibit a 80% mortality rate, with a high incidence of demyelinating brain lesions in survivors (6,16,19,20).

Previous experiments demonstrated that rapid correction of hyponatremia is followed by an initial decrease in brain water contents (after 2 to 24 h) (4,5,21). After 24 to 48 h, brain sodium and chloride contents increase to levels that exceed normonatremic values, accompanied by an increase in brain water contents (4,5). In contrast, the brain contents of organic osmolytes increase very slowly after correction, reaching normal levels only after 5 to 7 d (4,5).

These findings in nonazotemic animals are consistent with the results of previous studies of brain responses to hyponatremia and its correction (4,5). In this study, the brain contents of electrolytes (−25 to −29%) and organic osmolytes (−33 to −41%) in hyponatremic animals were significantly lower than those in control animals. Two hours after correction of hyponatremia with hypertonic saline solution, brain water contents were decreased (2.8% lower than control values); however, brain water and sodium contents had increased above control levels. In azotemic animals, in contrast, rapid correction of hyponatremia caused an initial decrease in brain water contents below control levels, but brain water, sodium, and potassium contents returned to near or below control levels by 24 h, with no overshoot.

The mechanism for and consequences of the overshoot of brain water and electrolyte contents after correction of hyponatremia remain hypothetical. Whether sodium and chloride (5) remain entirely in the extracellular space (thus provoking cellular dehydration) or enter cells (thus maintaining intracellular volume) is not known (4,5). Studies in normonatremic rats made hypernatremic suggested that increases in brain

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**Figure 1.** Changes in levels of major individual brain organic osmolytes after correction (2 or 24 h) of chronic (3-d) hyponatremia (H). C, control; gdw, grams dry weight. For each osmolyte, the postcorrection levels were significantly higher in the azotemic groups (P < 0.0001).

**Figure 2.** Comparison of total brain organic osmolyte contents (n = 25) between azotemic and nonazotemic rats after correction of chronic hyponatremia. gdw, grams dry weight. ■, azotemic; □, nonazotemic.
sodium and chloride contents are associated with expansion of the intracellular fluid compartment (22). Excessive brain hydration cannot be explained merely on the basis of compensatory overshooting of brain electrolyte levels to offset the depletion of organic osmolytes. The increase in brain sodium, chloride, and water contents could be the consequence of brain lesions induced by rapid correction of hyponatremia.

As previously described (4,5), we observed in this work that the brains of nonazotemic rats remained significantly depleted of organic osmolytes 24 h after correction of hyponatremia. Levels of myo-inositol and taurine (two of the most abundant brain osmolytes in mammalian brains) (1,23,24) remained at 41 and 50% of control values, respectively, 24 h after correction. However, the studies presented here demonstrate that, in azotemic animals, brain organic osmolytes are reaccumulated extremely rapidly after correction of chronic hyponatremia. Within 2 h, major osmolyte levels increased to 95% of control values in azotemic rats, whereas the levels of these solutes remained decreased (68% of control values) in nonazotemic animals; brain myo-inositol levels completely normalized and brain taurine levels reached 75% of control values in the azotemic group. By 24 h after correction, azotemic rats had completely reaccumulated their brain organic osmolyte contents. To our knowledge, such rapid large in vivo adaptive responses to increasing serum sodium concentrations have not been previously reported.

Hypertonicity induces synthesis of the sodium myo-inositol transporter (SMIT) via a recently identified transcription factor (the osmotic response element-binding protein) (25,26). Increased concentrations of SMIT mRNA first appear in the brain 1 h after hypertonic stress (27). Increased SMIT protein levels appear later, and increased rates of inositol transport do not occur for several hours (28). A recent study with cultured kidney cells demonstrated that this transcription factor responds to graded changes in tonicity, ranging from hypotonicity to hypertonicity (29). In the study reported here, the brains of uremic animals completely reaccumulated myo-inositol within 2 h after the infusion of hypertonic saline solution. This finding suggests that transport systems were upregulated before the correction of hyponatremia, allowing a rapid influx of myo-inositol and other organic osmolytes when an osmotic stress was imposed. Glycerophosphorylcholine is the only osmolyte that has been demonstrated to increase in response to high urea levels in renal cell lines, although urea has been demonstrated to affect gene expression of several transcription factors in cultured renal cells (30–32). The specific mechanism for the increase in brain myo-inositol levels observed in azotemic animals after rapid correction of hyponatremia in our studies is unknown.

In these studies, brain water, electrolyte, and organic osmolyte contents (with the exception of urea) did not differ significantly in normonatremic animals with or without azotemia. In hyponatremic animals, brain taurine levels were significantly higher in azotemic versus nonazotemic groups. Similarly, in studies of the dialysis disequilibrium syndrome, no changes were observed in the brain osmolyte contents of uremic rats before or after dialysis (33–35). Because urea

<table>
<thead>
<tr>
<th>Osmolyte Contents (μmol/g dry brain weight)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
<th>Group VII</th>
<th>Group VIII</th>
<th>Group IX</th>
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<tbody>
<tr>
<td>Glycine</td>
<td>4.69±0.19</td>
<td>4.90±0.21</td>
<td>3.89±0.14</td>
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<td>Lactate</td>
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<td>Glycerophosphorylcholine</td>
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- Data are mean ± SEM. 
- P ≤0.05, compared with nonazotemic rats.
- P <0.01, compared with nonazotemic rats.

Table 4. Minor brain organic osmolytes contents

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[1433-1441, 2002] Osmyotes in Azotemia and Hyponatremia

diffuses slowly across the blood-brain barrier (with equilibration after 4 to 10 h), rapid changes in plasma urea levels provoke changes in brain water contents by changing the brain/plasma urea gradient (33–35). In our studies, plasma urea levels continued to increase after correction of hyponatremia and might be expected to promote rather than counteract the hypertonic stress created by hypertonic saline solution administration.

The prompt reaccumulation of myo-inositol, taurine, and other organic osmolytes in our azotemic model could play a crucial role in promoting tolerance to large abrupt increases in serum sodium concentrations. Astrocytes, which are important in the demyelinating process that follows excessive correction of hyponatremia, are known to actively accumulate myo-inositol (36). Quantitatively, the organic osmolyte concentration difference between the brains of azotemic and nonazotemic animals equals 17 mmol/L brain water. With the assumption that organic osmolytes are primarily intracellular solutes, the intracellular concentration difference could be estimated to be 22 mmol/L water; this concentration difference would theoretically offset an approximately 11 mM increase in serum sodium concentrations. Therefore, the osmotic effect of enhanced reaccumulation of organic osmolytes could, by itself, partially explain the resistance of azotemic brains to injury resulting from rapid correction of hyponatremia.

If the increase in brain electrolyte contents that follows rapid correction of hyponatremia in nonazotemic animals represents an increase in brain sodium (and chloride) contents, then this change could adversely affect macromolecular structure and function, membrane transport properties, and excitability and could be an important factor in the pathogenesis of myelinolysis. Rapid reaccumulation of organic osmolytes could promote the maintenance of neuronal and glial cell volume without intracellular accumulation of nonorganic solutes, avoiding increased electrolyte concentrations created by cellular dehydration. Nonperturbing osmolytes could thus prevent the deleterious effects of an abrupt increase in tonicity.

Whether the difference in brain organic osmolyte contents observed in azotemic rats is attributable to urea itself or to other components is not known. Other mechanisms are also probably involved, if we consider the very large increases in serum sodium concentrations (>40 mEq/L) that are sometimes tolerated by azotemic rats. Urea by itself might have some protective properties that improve the tolerance of azotemic brains to osmotic stress. Urea has been demonstrated to protect renal medullary cells against the proapoptotic effects of hyperosmotic stress induced by sodium chloride (37). The plasma urea of dogfish has been demonstrated to exert cardioprotective effects against oxidative stress (38). Perfusion of isolated hearts with high concentrations of urea has also been demonstrated to protect tissue against oxidative injury (38). Protection against oxidative injury may be important in hyponatremia, because it is known that hyponatremic brain tissue is depleted of glutathione and other organic osmolytes with antioxidant properties (39).

Organic osmolytes play an osmoprotective role throughout nature (40,41). The compounds that permit mammalian brains to adapt to changes in serum sodium concentrations are also used by marine invertebrates as they adapt to changes in the salinity of their environment. Our observation that urea protects animals against osmotic brain injury is phylogenetically consistent. Elasmobranchs (sharks, skates, and rays) that thrive in sea water exhibit high plasma urea concentrations (approximately 400 mM) (40,41). Their high plasma urea concentrations make it possible for these organisms to avoid exposure of their cells to the deleterious effects of the high salt concentrations of their marine environment.

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References
