The Role of Organic Osmolytes in the Cerebral Cell Volume Regulatory Response to Acute and Chronic Renal Failure

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ABSTRACT
Brain cells respond to increased osmolality of the extracellular fluid by accumulating inorganic electrolytes and nonperturbing organic osmolytes to limit the extent of brain cell shrinkage. It is unclear whether urea is an effective osmole that triggers this adaptive response. Therefore, the amount of brain water and the cerebral content of organic osmolytes in rats with acute renal failure induced by bilateral ureteral ligation and in animals with chronic renal failure for 6 weeks created by a 75% reduction in renal mass were measured. Eight hours after the onset of acute renal failure, the BUN concentration and serum osmolality were 61 ± 4 mg/dL and 314 ± 2 mosmol/kg, respectively, compared with 13 ± 1 mg/dL and 288 ± 1 mosmol/kg, respectively, in sham-operated rats. This hyperosmolal state reduced brain water from 79.7 ± 0.1% in controls to 79.2 ± 0.1% in uremic animals (P < 0.01). During acute renal failure lasting 48 h, the BUN concentration and serum osmolality rose to 251 ± 10 mg/dL and 370 ± 4 mosmol/kg versus 18 ± 1 mg/dL and 286 ± 4 mosmol/kg, respectively, in sham-operated control rats. The percentage of brain water content was unchanged in rats with azotemia for 48 h—79.6 ± 0.1 compared with 79.5 ± 0.2% in controls. Stabilization of brain volume was associated with a 19% increment in total organic osmolyte content in brain cells from 123 ± 6 to 146 ± 2 mmol/kg dry wt (P < 0.05). In rats with chronic renal failure, there was an increase in the BUN concentration from 22 ± 1 in controls to 64 ± 7 mg/dL, in association with a rise in serum osmolality from 289 ± 1 to 311 ± 4 mosmol/kg. There was no difference in the brain water content—79.1 ± 0.1 versus 78.9 ± 0.2% in animals with chronic renal failure or control rats, respectively. Furthermore, there was only a 7% rise in total brain organic osmolytes in rats with sustained renal insufficiency—158 ± 10 compared with 148 ± 7 mmol/kg dry wt in the animals with intact kidneys. It was concluded that acute renal failure is a hyperosmolal state and that urea is an effective osmole that causes transient brain cell shrinkage. Until equilibrium is reached across brain cell membranes, an acute elevation in the serum urea concentration activates the cerebral cell volume regulatory mechanism, leading to the adaptive accumulation of organic osmolytes.

Key Words: Cerebral cell volume regulation, organic osmolytes, acute and chronic uremia, hyperosmolality

Maintenance of cell volume within narrow limits is a vital biologic function of all organisms (1,2). This task is especially important in the mammalian brain because it poorly tolerates alterations in cell size after the imposition of an osmolar gradient between the intracellular and extracellular water compartments (3,4). Therefore, brain cells have developed the capacity to modulate the cytoplasmic concentration of inorganic electrolytes and an array of organic osmolytes in direct response to fluctuations in plasma osmolality (1,3). This adaptation limits water egress from cells and prevents the deleterious consequences of cerebral cell shrinkage.

There has been renewed interest in the past several years in the identification of the major classes of cerebral organic osmolytes and elucidation of the mechanisms involved in their accumulation within cerebral cells during hyperosmolar states (5–7). The brain content of these compatible solutes is increased during hypernatremia and hyperglycemia (1,3). However, it is unclear whether an elevated plasma urea concentration provokes the same adaptive increment...
in organic osmolytes in cerebral cells. Therefore, we conducted the following experiments to determine whether organic osmolytes accumulate within the brain in rats with acute and chronic renal failure.

EXPERIMENTAL METHODS AND STUDY DESIGN

Animals

Male Sprague-Dawley rats were purchased from Taconic Farms (Germantown, NY). Animals were housed in a facility with a 12-h light-dark cycle and an ambient temperature of 25°C. Rats were fed standard rodent chow containing 22% protein (Ralston Purina, St. Louis, MO) and provided tap water to drink ad libitum. Pair feeding was not performed because the pattern of weight gain was similar in the experimental and control animals subjected to the acute renal failure (ARF) and chronic renal failure (CRF) protocols. Rats weighed 170 to 240 g at the beginning of the studies.

Disease Models

Animals were anesthetized with pentobarbital, 45 mg/kg body wt. administered as an ip injection. In order to induce ARF, a midline incision was made and two ligatures were placed around both ureters between the renal pelvis and the bladder. Sham-operated controls underwent the same procedure, including manipulation of the ureters. The surgical wound was closed in two layers, and the rats were maintained for 8 to 48 h.

Production of CRF was accomplished in a one-step procedure by the performance of a right nephrectomy and the removal of the bulk of the kidney tissue at the upper and lower poles of the left kidney with a scalpel. Hemostasis was achieved by the placement of Gelfoam (Upjohn, Kalamazoo, MI) on the cut surface of the kidney. The amount of renal parenchyma removed was estimated by the following formula:

\[
\text{% reduction} = \frac{\text{right kidney weight + weight of removed left kidney}}{2 \times \text{right kidney weight}}
\]

Sham-operated control rats were subjected to comparable surgery, including manipulation of both renal pedicles. Animals in the CRF protocol were maintained for 6 wk. Two days before the rats were euthanized, the animals were placed in individual metabolic cages (Nalge, Rochester, NY) with free access to water but not food. A urine sample was collected for 24 h to enable determination of endogenous creatinine clearance and urinary protein excretion.

Procurement of Samples

At the completion of the ARF and CRF experimental studies, rats were killed by decapitation. A blood sample was obtained for the measurement of serum osmolality, Na⁺, creatinine, and BUN concentrations. The brain was rapidly excised, and a portion (100 to 300 mg) was removed and desiccated to a constant weight for 3 days at 70°C to calculate the percentage of brain water content. The remaining brain tissue (1 to 1.3 g) was homogenized in 3 to 5 mL of ice-cold 6% perchloric acid with a Dounce homogenizer (Wheaton, Millville, NJ) and was placed on ice for 30 min. The sample was centrifuged at 12,000 × g for 15 min, and the supernatant was removed and neutralized to pH 7.2 to 7.6 with 1 N HCl or 1 N KOH. Specimens were saved at −70°C until a batch assay of the organic osmolytes was performed.

Analytical Methods

The serum Na⁺, creatinine, and BUN and urinary creatinine concentrations were determined with an automated analyzer (Beckman, Fullerton, CA). Serum osmolality was measured with a vapor pressure osmometer (Model 5100C; Wescor, Logan, UT). Urinary protein concentration was measured with the Coomassie blue binding reagent (Bio-Rad, Richmond, CA).

Organic osmolytes were measured by HPLC as described previously (8). Briefly, 2 mL of each neutralized perchloric acid extract was filtered serially through a C-18 column (Bond Elut; Varian, Palo Alto, CA) and a 0.45-μm syringe filter (HV Type; Millipore Corp., Bedford, MA). An aliquot (0.8 mL) of the filtrate was lyophilized, reconstituted with 0.4 mL of mobile phase (0.005% calcium disodium EDTA), and centrifuged at 5,000 × g for 5 min. The supernatant (0.1 mL) was injected into a Waters HPLC system (Waters Associates, Milford, MA), fractionated on a Sugar Pak I column (Waters), and analyzed with a refractive index detector. The flow rate was 0.6 mL/min, and the column temperature was 92°C. Osmolyte quantitation was performed by analysis of peak areas and comparison to known standards.

Glutamate could not be analyzed by HPLC analysis so it was measured by a standard spectrophotometric assay with the generation of NADH by glutamate dehydrogenase (9). All reagents for this assay were purchased from Sigma Chemical Co. (St. Louis, MO).

Statistical Methods

The differences in the data for the experimental and control groups in the ARF and CRF studies were analyzed by t test, with the Bonferroni correction for multiple comparisons. The brain content of organic osmolytes is expressed as millimoles per kilogram.
The BUN and serum creatinine concentrations were
TABLE I.
the amount accrued by the sham-operated controls
control animals.
proteinuria, 244 ± 44 versus 28 ± 3 mg/24 h in
animals with ARF for 48 h.
control animals. Serum osmolality was elevated from
dry tissue weight. The results are provided are mean ± SE.
RESULTS
The initial and final body weights were similar in
the experimental rats with ARF for 48 h (N = 5); 226 ± 4 and 212 ± 6 g, and the control rats (N = 6); 222 ± 2 and 220 ± 4 g (Table 1). Animals with bilateral ureteral ligation manifested decreased spontaneous activity but were more irritable than control counterparts. The serum Na⁺ concentration was 125 ± 1 versus 133 ± 1 mmol/L in ARF and control rats, respectively (P < 0.005). As expected, the BUN and serum creatinine levels were markedly higher in uremic rats, 251 ± 10 and 6.6 ± 0.1 mg/dL, compared with 18 ± 1 and 0.33 ± 0.03 mg/dL in sham-operated control animals. Serum osmolality was elevated from 286 ± 3 in control rats to 370 ± 4 mosmol/kg in animals with ARF for 48 h.
The renal ablative procedure resulted in a loss of
78.4 ± 1.9% of the total kidney parenchymal mass. Rats with CRF had a reduced GFR, 0.15 ± 0.02, estimated by the endogenous creatinine clearance, compared with sham-operated animals, 0.40 ± 0.02 mL/min/100 g body wt. Uremic rats had significant proteinuria, 244 ± 44 versus 28 ± 3 mg/24 h in control animals.
The initial body weight in the rats with CRF (N = 5) was 206 ± 6 g, and the final weight 6 wk later was 348 ± 13 g (Table 1). This weight gain was less than the amount accrued by the sham-operated controls (N = 6), whose starting weight was 212 ± 12 g and whose final weight was 423 ± 20 g (P < 0.02). There was no discernible difference in behavior displayed by these two groups of animals during the entire study period. The serum Na⁺ concentration was identical, 141 ± 1 mmol/L, in the CRF and control rats. The BUN and serum creatinine concentrations were 64 ± 7 and 1.6 ± 0.2 mg/dL, respectively, in the CRF rats compared with values of 22 ± 1 and 0.53 ± 0.03 mg/dL, respectively, in sham-operated animals. CRF resulted in an 8% elevation in serum osmolality from 289 ± 1 in control rats to 311 ± 4 mosmol/kg; however, the magnitude of the hyperosmolal state was significantly less than in rats with ARF for 48 h.
Brain water content was unaffected by ARF for 48 h or CRF (Table 1). Thus, in rats with bilateral ureteral ligation, the percentage of brain water was 79.6 ± 0.2 versus 79.5 ± 0.2% in control animals. Similarly, in animals with renal ablation, the percentage of brain water was 79.1 ± 0.1 versus 78.9 ± 0.2% in sham-operated rats.
In order to determine whether the elevation in serum urea concentration earlier in the course of ARF causes cerebral cell shrinkage, brain water content was measured in rats with ARF lasting 8 h (Table 2). Animals with bilateral ureteral ligation (n = 5) had a BUN concentration and serum osmolality of 61 ± 4 mg/dL and 314 ± 2 mosmol/kg, respectively, after 8 h, compared with 13 ± 1 mg/dL and 288 ± 1 mosmol/kg in sham-operated control rats (N = 4). This hyperosmolal state resulted in a significant reduction in brain water content from 79.7 ± 0.1% in control rats to 79.2 ± 0.1% in animals with ARF (P < 0.01).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (N = 6)</th>
<th>48-h ARF (N = 5)</th>
<th>Control (N = 6)</th>
<th>CRF (N = 5)</th>
</tr>
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<tbody>
<tr>
<td>Weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>222 ± 2</td>
<td>226 ± 4</td>
<td>212 ± 12</td>
<td>206 ± 6</td>
</tr>
<tr>
<td>Final</td>
<td>220 ± 4</td>
<td>212 ± 6</td>
<td>423 ± 20</td>
<td>348 ± 13³</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>18 ± 1</td>
<td>251 ± 10⁴,a</td>
<td>22 ± 1</td>
<td>64 ± 7³</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.3 ± 0.01</td>
<td>6.6 ± 0.1⁵,b,c</td>
<td>0.51 ± 0.03</td>
<td>1.6 ± 0.2⁶</td>
</tr>
<tr>
<td>Serum Na⁺ (mmol/L)</td>
<td>133 ± 1</td>
<td>125 ± 1³,b,c</td>
<td>141 ± 1</td>
<td>141 ± 1</td>
</tr>
<tr>
<td>Serum Osmolality (mosmol/kg)</td>
<td>286 ± 3</td>
<td>370 ± 4³,b,c</td>
<td>289 ± 1</td>
<td>311 ± 4⁶</td>
</tr>
<tr>
<td>Brain Water (%)</td>
<td>79.5 ± 0.2</td>
<td>79.6 ± 0.2</td>
<td>78.9 ± 0.2</td>
<td>79.1 ± 0.1</td>
</tr>
</tbody>
</table>

⁴ P < 0.001 versus respective control group.
⁵ P < 0.001 versus CRF.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (N = 4)</th>
<th>8-h ARF (N = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>238 ± 3</td>
<td>244 ± 5</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>13 ± 1</td>
<td>61 ± 4⁴</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.4 ± 0.0</td>
<td>1.56 ± 0.08⁴</td>
</tr>
<tr>
<td>Serum Na⁺ (mmol/L)</td>
<td>142 ± 1</td>
<td>140 ± 1</td>
</tr>
<tr>
<td>Serum Osmolality (mosmol/kg)</td>
<td>288 ± 1</td>
<td>314 ± 2⁴</td>
</tr>
<tr>
<td>Brain Water (%)</td>
<td>79.7 ± 0.1</td>
<td>79.2 ± 0.1⁴</td>
</tr>
</tbody>
</table>

⁴ P < 0.01 versus control group.
The brain content of urea and the organic osmolytes that were surveyed in these experiments are summarized in Table 3. In rats with ARF for 48 h, the cerebral urea content was elevated 6.4-fold compared with that of sham-operated controls. The brain content of taurine, glutamine, glutamate, creatine, and myo-inositol was enhanced 17, 10, 7, 12, and 26%, respectively, above the levels observed in sham-operated animals. The increment in the cerebral content of the organic osmolytes in ARF lasting 48 h was significant for all of the solutes except glutamate. The glycerophosphorylcholine (GPC) level was very low (<1 mmol/kg dry wt) in cerebral tissue examined from normal rats and declined by 56% during the 48-h interval of ARF. The total brain cytosolic pool of organic osmolytes increased by 19% from 123 ± 6 in control rats to 146 ± 2 mmol/kg dry wt in ARF animals.

During the course of CRF, the brain content of urea was 100% higher than the value detected in control rats. The cerebral content of taurine, glutamine, glutamate, creatine, and myo-inositol rose only 10, -4, 9, 5, and 9%, respectively, in rats with reduced kidney mass versus sham-operated animals. GPC content fell by 15% in the CRF rats. In animals with sustained renal insufficiency, the sum of the organic osmolytes, 158 ± 10 mmol/kg/dry wt, rose only 6.8% above the value detected in the brain tissue of the control rats, 148 ± 7 mmol/kg dry wt. Furthermore, the increment in the total cerebral content of organic osmolytes in CRF rats was significantly smaller than the 19% elevation observed in the animals with ARF lasting 48 h (P < 0.05).

DISCUSSION

The results of these experiments indicate that acute azotemia is an effective hyperosmolar state that activates the cerebral cell volume regulatory mechanism. This adaptive response results in the accumulation of organic osmolytes to minimize contraction of the cerebral intracellular water compartment. In contrast, during CRF, despite persistent hyperosmolality of the extracellular fluid, there is neither a perturbation in brain cell size nor an increment in the cerebral cytosolic content of organic osmolytes. Prolonged uremia enabled the equilibration of urea across brain cell membranes and the restoration of normal cerebral cell size. This abrogated the activation of the cerebral cell volume regulatory adaptation.

There was an unequivocal difference in the degree of renal impairment and the magnitude of the hyperosmolar stress between the rats with ARF for 48 h and the animals with CRF. However, our data in the rats with ARF for 8 h indicate that the duration of uremia is the primary factor that determines whether an elevated plasma urea concentration causes brain cell shrinkage and activates the cerebral cell volume regulatory mechanism. The degree of azotemia in rats with CRF represented an increment in plasma osmolality sufficient enough to cause egress of water from brain cells. Despite comparable increases in BUN concentration and plasma osmolality in rats with ARF for 8 h and animals with CRF, only the former group displayed a reduction in brain water content. Thus, the acuity of uremia rather than its severity accounts for the accumulation of organic osmolytes in the brain of rats with ARF and not CRF. We acknowledge that the accumulation of organic osmolytes during ARF for 48 h may represent a response to the azotemia per se and not the associated cell volume contraction.

ARF causes severe disturbances in brain cell Na⁺ transport and alterations in cytosolic Na⁺ concentration that may interfere with the accumulation of organic osmolytes within cerebral cells (10). Furthermore, in these experiments, ARF was associated with mild hyponatremia. In vitro studies with synaptosomes isolated from rats with hyponatremia indicate diminished cerebral uptake of taurine, a representa-
tive osmoprotective molecule (11). Despite these confounding considerations, which might be predicted to reduce the cerebral accumulation of organic osmolytes, the brain content of organic osmolytes was enhanced during 48-h ARF.

The brain is thought to respond in a uniform manner to hyperosmolar states arising from an elevated plasma concentration of endogenous solutes by initially increasing the cytosolic pool of inorganic electrolytes, followed by an accumulation of organic osmolytes (1,2,12). The cell shrinkage induced by an acute elevation in the effective plasma osmolality causes a rise in cytosolic ionic strength that triggers changes in ion conducting and transporting pathways (12,14). This is followed by a gradual increase in organic osmolyte content, culminating in the restoration of brain cell size towards normal (14). This patterned adaptation occurs during hypertremia of 48- to 96-h duration and hyperglycemia lasting for 1 week (1,3). Acute uremia can be added to the list of clinical conditions that activates the cerebral cell volume regulatory response. However, in contrast to the response to hypertremia or hyperglycemia, the brain content of electrolytes does not increase during ARF (15,16). Our data indicate that ARF is a dynamic condition characterized by steadily worsening azotemia and a gradient for urea across cerebral cell membranes. Organic osmolytes are recruited to temporarily fill the gap between the sum of the urea and electrolyte concentrations in the extracellular and intracellular fluid compartments until the equilibration of urea is achieved across the cerebral cell membrane, as seen in CRF.

It is precisely because inorganic electrolytes have been demonstrated not to play a substantial role in cerebral cell volume regulation in uremic states that we chose to focus on the organic osmolytes (15–17). The magnitude of the increase in total brain organic osmolyte content is similar in hypertremia, hyperglycemia, and ARF, i.e., 15 to 20%. The disparity in the brain content of total organic osmolytes in the two control groups probably resulted from acute nutritional factors in the sham-operated rats included in the ARF protocol or differences in animal age at the conclusion of the two sets of experiments.

A comparison of the measured sum of the cerebral organic osmolytes and the plasma osmolality in animals with ARF for 48 h suggests that the increment in the brain cell content of the osmoprotective solutes is insufficient to balance the rise in serum osmolality. We acknowledge this apparent discrepancy between calculated cerebral and plasma osmolality. The assays used in these studies should have detected all known cerebral organic osmolytes. However, there may be other as-yet-unidentified organic osmolytes that contribute to brain cell volume regulation during azotemia. One candidate molecule could be ammonium carbamate, the synthesis of which should be enhanced during acute uremic states (18) and for which there is a membrane transport system to facilitate cell uptake (19). In any event, this would not detract from our conclusion that ARF, but not CRF, triggers the accumulation of organic osmolytes in brain cells.

Urea is considered an ineffective osmole that does not cause significant cell shrinkage compared with Na⁺ or glucose in the absence of insulin (1,3). However, the reflection coefficient and the permeability: lipid solubility ratio of urea are similar to the values for mannitol, glycerol, and sucrose (4). Lien et al. reported a reduced brain:plasma urea ratio in normal and azotemic rats (6). The infusion of urea into animals results in a transient decline in cerebrospinal fluid pressure, lasting 4 to 6 h, as the brain shrinks away from the skull (3). Urea has been administered parenterally to decrease cerebral edema in select clinical circumstances. The protective effect of urea infusion against the development of central pontine myelinolysis during the rapid correction of hyponatremia has been attributed to the gradual entry of urea into brain cells and the supplementation of the cytosolic pool of solutes (20). Finally, the dialysis disequilibrium syndrome in rats has been attributed to delayed egress of urea from the cerebral cells in the face of a rapid dialysis-induced fall in the plasma urea concentration, leading to brain swelling (16). All of these data suggest that urea is not freely permeable across the brain cell membrane and that acute alterations in BUN result in a plasma-to-brain concentration gradient for urea. Therefore, it is not surprising that severe uremia lasting 48 h activates the cerebral cell volume regulatory adaptation and that we observed significant accumulation of organic osmolytes under these conditions.

The response of brain cells to acute uremia differs from that of cultured renal cells. MDCK cells and GRB-MAL1 and PAP-HT25 cells which are derived from rabbit renal inner medullary epithelia, accumulate more organic osmolytes when media are made hypertonic by the addition of NaCl or raffinose than by urea (21,22). Urea induces only GPC accumulation and tends to inhibit the accumulation of other organic osmolytes in PAP-HT25 cells (22). Hypertonic saline and raffinose, but not urea, provoke an adaptive increase in aldose reductase activity and sorbitol accumulation in GRB-PAP1 cells (23). This reflects the rapid diffusion of urea across the renal cell membrane and the absence of any cell volume change. In contrast, because of the unique anatomic feature of brain capillaries, namely, the tight junctions between adjacent endothelial cells that constitute the blood brain barrier, water permeability exceeds urea permeability and creates a nonequilibrium state for urea concentration between brain cells and the
plasma. This accounts for the different patterns of accumulation of organic osmolytes by cerebral cells in vivo and by renal cells in vitro in response to hyperosmolar states (3,4).

The increment in organic osmolytes in renal cells during hyperosmolar states is achieved by enhanced DNA transcription and the synthesis of new transporters that mediate the uptake of osmoprotective organic molecules (12,14). We have demonstrated that the uptake of taurine, an index organic osmolyte, is increased in synaptosomes isolated from the brain of rats with hypernatremia, hyperglycemia, and ARF, but not animals with CRF (24–27).

The organic osmolytes have been divided into two classes: (1) nonperturbing, compatible molecules whose cytosolic concentration can be raised during hyperosmolar states without causing destabilization of protein structure and function; and (2) counteracting solutes whose activity antagonizes the deleterious effect of selective molecules on cell metabolism (13). The intracellular content of methyamines, and in particular GPC, has been documented to rise preferentially in cultured renal medullary cells and in the inner medulla and papillary regions of the kidney in direct relationship to the external urea concentrations (22,28,29). It is apparent from our results that brain cells do not contain large quantities of GPC under normal conditions. In addition, its concentration in cerebral cells is not elevated enough to attenuate the deleterious effect of acute uremia on neuronal function by counteracting the adverse effects of increased brain cell urea content.

In conclusion, we have demonstrated that ARF is a hyperosmolar state that causes transient brain cell shrinkage and subsequent activation of the cerebral cell volume regulatory adaptation. This results in the accumulation of organic osmolytes in brain cells. The magnitude of this response is comparable to that achieved during hypernatremia and hyperglycemia of similar duration. In contrast, CRF is not a significant osmolar stress that causes perturbation in cerebral cell size, and therefore, prolonged uremia does not stimulate the build-up of organic osmolytes within brain cells.

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