Treatment of hyponatraemia by urea decreases risks of brain complications in rats. Brain osmolyte contents analysis

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Abstract

Background. Brain damage (myelinolysis) develops in hyponatraemia after a large increase in serum sodium regardless of the currently available methods of correction. However, a preliminary study suggests that treatment of hyponatraemia with urea limits the risks of brain lesions in rats. Benefits of sustained high blood levels of urea and mechanisms of protection remain hypothetical.

Methods. In the first part of the study, hyponatraemic rats received repeated (i.p.) doses of urea (2 g/kg b.w./6 h) leading to sustained blood levels (urea ± 230 mg/dl). Neurologic outcome was compared to correction of hyponatraemia by water diuresis. In the second part of the study, we analysed the adaptive response of the brain to correction of hyponatraemia with either urea or water diuresis, by measurement of cerebral osmolyte contents.

Results. Despite a large correction of the serum sodium (mean Δ SNa = 32 mEq/l/24 h), mortality rate (13%) and neurological symptoms were low in the urea-treated group contrary to control groups treated by water diuresis (mortality: 87%). This shows with stronger evidence the protective effect of urea against brain myelinolysis. In the second part, analysis of brain composition shows that, in the urea groups, 24 h after correction, intracerebral hyperionization (NaCl) was avoided and brain water contents remained normal. No significant changes of the major brain organic osmolyte composition were observed after urea administration except reaccumulation of betaine. No difference in brain composition was noted regarding concomitant plasma urea levels (> or <150 mg/dl). In rats treated by water diuresis, the brain remained also significantly depleted in organic osmolytes 24 h after correction, but contrary to administration of urea, this treatment was associated with a high mortality rate.

Conclusions. These comparative results suggest a specific brain-protective effect of urea itself against myelinolysis.

Keywords: brain; hyponatraemia; osmotic demyelinating syndrome; urea

Introduction

Chronic hyponatraemia, when rapidly corrected, leads to brain myelinolysis and often irreversible neurological damage [osmotic demyelination syndrome (ODS)] [1]. Myelinolysis develops regardless of the methods used to correct the serum sodium with the exception of urea: correction with urea significantly reduces the brain damage as shown in a preliminary study in rats [1,2]. Urea is currently being used, and has been for years, in our hospitals to treat hyponatraemic patients [1,3,4]. There is also clinical evidence suggesting that uraemic patients tolerate large fluctuations in serum sodium without development of ODS [5].

Slow re-establishment of brain organic (non-perturbing) osmolyte contents is one of the major proposed mechanisms implicated in the pathogenesis of the demyelinating process [1,6]. The inability of the hyponatraemic brain to counteract an acute and large increase in serum sodium leads to excessive brain dehydration with subsequent cerebral injury [1,6,7].

We demonstrated recently in hyponatraemic rats that concomitant azotemia (48 h) protects the brain against neurological damage and death after overcorrection [8]. Subsequently, we showed in this model that the brain of azotemic rats is able to reaccumulate very rapidly (within 2 h) organic osmolytes (myo-inositol, taurine, glutamine, ...), normalizing completely their intracerebral contents 24 h after correction of the
serum sodium [9]. In non-azotemic animals, the reuptake of organic solutes takes 5–7 days to be achieved [1,6]. The definite factor responsible for this unique cerebral response to osmotic stress observed in our azotemic model remains unknown. An increase of organic osmolyte levels implicates previous synthesis and rapid activation of specific transporters, e.g. sodium-myo-inositol co-transporter (SMIT) for myo-inositol, via transcription factors (e.g. osmotic response element binding-protein) [10], establishing the link between the environmental osmotic stimuli and the cellular adaptive response.

In our initial experiments [2] both the incidence and the severity of brain lesions were substantially lower with exogenous urea administration than with correction by hypertonic saline [1,2]. Thus, we showed that chronic hyponatraemic rats with azotemia tolerate a large correction of serum sodium [8], this led us to evaluate the effect of higher and more sustained blood urea levels after exogenous urea administration on the neurological outcome.

We confirmed that the use of exogenous urea provides striking protective properties for the brain.

Parallely, we explored in this experimental model, the potential influence of urea on brain composition during correction of hyponatraemia and compared this cerebral adaptive response to correction with water diuresis.

We showed that the adaptive response of urea-treated brains differs from the azotemic model [9], suggesting the existence of more specific protective mechanisms for urea.

Materials and methods

Animals

We used male Wistar rats (Charles-River Laboratories, Brussels, Belgium) weighing 250–350 g for all studies. The rats were housed in individual cages for at least 3 days before starting the experiments.

They were maintained on a standard diet of pelleted chow and were given free access to water. The mean temperature was controlled at 25°C, with lights on from 7:00 a.m. to 7:00 p.m.

Induction of hyponatraemia

Chronic (3 days) severe hyponatraemia (serum sodium concentration <120 mEq/l) was induced by continuous infusion of synthetic vasopressin via subcutaneous osmotic pumps (Alzet model 2001; Palo Alto, CA), at a rate of 24 mU/h and intraperitoneal (i.p.) injections of 2.5% (140 mM) D-glucose in water. The 2.5% D-glucose was given at a dose equivalent to 5% of body weight twice daily (9:00 a.m. and 5:00 p.m.) on days 1 and 3, and once (at noon) on day 2. The rats had no access to food or water during the induction phase.

Correction of hyponatraemia. Rats were randomized to receive either urea or to be treated by water diuresis. In the groups treated with urea (group 1 in experiment 1 and ‘Urea’ in experiment 2), rats received on day 4 i.p. injections of urea 24% (urea powder diluted in 5% D-glucose in water freshly prepared each time).

Urea was administered i.p. over 5 min at a dose of 0.8 ml/100 g body weight every 6 h during 24 h (8 g/kg b.w./day) (7:00 a.m., 1:00 p.m., 7:00 p.m., 1:00 a.m.).

In the group treated by water diuresis (group 2), the osmotic minipumps were withdrawn on day 4. In both models, rats were maintained with no access to food and water during the correction period (day 4).

Access to food was free from day 5. Access to water was limited to 10 ml during the first 12 h of day 5 in order to avoid spontaneous relowering of the serum sodium due to exacerbated water avidity.

Blood measurements

Blood samples (0.25 ml) were collected via tail transection, with light ethyl ether anaesthesia, for serum sodium and urea measurements (MODULAR p800, Roche).

Brain composition study

In experiment 2, rats were decapitated on day 5 (24 h after correction with urea) in the group treated with exogenous urea in order to determine brain water electrolytes and organic osmolyte composition.

Brains were weighted, bisected and one hemisphere reweighed for calculation of brain water contents. Hemispheres were immediately frozen and stored at −70°C for subsequent analysis.

Analytic procedures

Detailed procedure for brain water, electrolytes and organic osmolytes has been described previously [9] and are briefly reported here.

Brain water and electrolytes

One hemisphere of each brain was dried at 100°C for 48 h and reweighed for brain water content determination. Dried tissue was crushed and dissolved in 0.75 N HNO3 for sodium and potassium measurement by flame photometry.

Brain organic osmolyte contents

Frozen brain was crushed under liquid nitrogen and lyophilized. Myo-inositol, urea, betaine, glycerophosphorylcholine and creatine were determined by HPLC (Waters) using a Sugar Pak I column [11]. Metabolites were detected and quantified at a Waters Millenium 32 chromatography workstation interfaced with Empower.

Aminoacid content was determined by HPLC after derivitization with phenylisothiocyanate (Picotag column, Waters Corporation, Milford, MA) [12].

Experimental groups

Experiment 1. In this first set of experiments, we evaluated the effect of rapid correction of chronic (3 days) hyponatraemia with exogenous urea (group 1, n=15) on the
neurological outcome and survival and compared this group to correction by water diuresis (group 2, \( n = 16 \)). Experimental protocol is described above. After correction (day 4), rats were observed and behavioural abnormalities and deaths were recorded up to day 10. Rats were reported to present neurological symptoms or not. Typical neurological symptoms include gait disturbance (ataxia), hyperexcitability (rats jumping all over the cages), spasticity of the extremities, paralysis of the limbs, marked lethargy to the point of unresponsiveness [2].

The number of surviving rats on day 10 was compared between both groups. Serum sodium and urea levels were measured on day 0, day 4 (hyponatraemia) and 12 h, 24 h and 36 h after correction with either urea or water diuresis.

**Experiment 2.** In this experiment, we evaluated the effect of rapid correction of chronic (3 days) hyponatraemia by exogenous urea or water diuresis on brain adaptative mechanisms. A series of 26 rats was treated with urea and 17 animals were corrected by water diuresis as described above. Rats were decapitated 24 h after correction for brain analysis following the methods cited above.

**Statistical methods**

Data are given as mean ± SE. Differences in plasma and mortality rate between groups in experiment 1 were assessed by \( t \)-test and chi-square tests as appropriate. In experiment 2, differences were assessed by one-factor ANOVA with significance determined by Scheffe F-test (512/613). Significance was accepted at \( P < 0.05 \) level.

This study was approved by the ethical comity for experimental research of the Faculty of Medicine, Free University of Brussels.

**Results**

**Experiment 1 (Table 1)**

The results of the kinetics of serum sodium and urea values and outcome in each group are presented in Table 1. All of the rats developed severe (SNa <120 mEq/l) hyponatraemia without significant differences between groups. Control of serum sodium at 36 h in the two groups showed no secondary decrease in the natraemia (\( \Delta \text{SNa} = 32 ± 3 \text{ mEq/l}, \text{ range 27–39 meq/l} \) after refeeding and free access to water.

In group 1, the i.p. administration of urea leads to approximately a 5-fold increase in the urea blood levels after 12 h and a 10-fold increase after 24 h. Blood urea levels were >100 mg/dl in all except three rats (59, 77 and 87 mg/dl at 24 h). No behavioural changes were associated with the azotemia.

As shown in Table 1, the administration of urea produced a large gradient of serum sodium correction (\( \Delta \text{SNa} = 32 ± 0.8 \text{ mEq/l/24 h} \), above the toxic threshold for rat brain (20–25 mEq/l/24 h) [2]. The serum sodium increased progressively during the 24 h of correction with a \( \Delta \text{SNa} \) slightly higher in the last 12 h. The changes in serum sodium correction during the 24 h were not different in both groups (Table 1). Serum sodium values in the urea group are: 114 ± 1.7 (101–125) at 12 h and 133 ± 1.7 (119–144) at 24 h after correction.

During the following days, only 3 of 15 rats developed neurological signs typically associated with brain myelinolysis [2]. Two of them died before day 10. These two rats corrected their serum sodium by 33 mEq/l/24 h and 34 mEq/l/24 h, respectively, with concomitant blood urea levels of 350 mg/dl and 122 mg/dl. The 13 other rats (87%) fared well, remained asymptomatic up to day 10. Five of the 13 rats lost weight.

In group 2 (Table 1), serum sodium levels are: 116 ± 1.4 (107–130) at 12 h and 134 ± 1.1 (127–146) at 24 h. Despite similar correction levels (mean \( \Delta \text{SNa} = 31 ± 1 \text{ mEq/l/24 h} \), NS vs group 1), 94% of the animals exhibited neurological manifestations encountered in myelinolysis (\( P < 0.01 \) compared with group 1).

In this group, 87% (14/16) of the animals died before day 10 (\( P <0.01 \) compared to group 1). All rats lost weight. Comparison of these two treatment regimens indicates a significantly lower incidence of neurological damage and death when correction of hyponatraemia was obtained with urea than with water diuresis.

**Experiment 2 (Tables 2–5)**

In the urea and water diuresis groups intended to be studied for brain composition, all rats developed severe

<table>
<thead>
<tr>
<th>Table 1. Serum sodium, urea levels and neurological outcome after correction of hyponatraemia with urea or water diuresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
</tr>
<tr>
<td>---------</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Urea</td>
</tr>
<tr>
<td>(n = 15)</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>diuresis</td>
</tr>
<tr>
<td>Range</td>
</tr>
</tbody>
</table>

SNa: serum sodium (mEq/l); \( \Delta \text{SNa} \): gradient of serum sodium correction; urea: mg/dl.

\* \( P < 0.001 \) compared with group 2. Data are mean ± SE.
Data are mean ± SE.

Table 3. Brain water and electrolyte contents 24 h after correction of hyponatraemia in rats treated with exogenous urea compared to water diuresis (WD)

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 10)</th>
<th>Hyponatraemia (n = 16)</th>
<th>WD (n = 17)</th>
<th>Urea (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain water content (litres H₂O/kg dry brain weight)</td>
<td>3.46 ± 0.02</td>
<td>3.64* ± 0.03</td>
<td>3.55* ± 0.02</td>
<td>3.42** ± 0.02</td>
</tr>
<tr>
<td>Na⁺ content (μEq/g dry brain weight)</td>
<td>192 ± 1.8</td>
<td>157 ± 1.5</td>
<td>219* ± 3.17</td>
<td>202** ± 2.5</td>
</tr>
<tr>
<td>K⁺ content (μEq/g dry brain weight)</td>
<td>452 ± 4.2</td>
<td>380 ± 3.1</td>
<td>457 ± 3.9</td>
<td>440** ± 3.6</td>
</tr>
<tr>
<td>Brain weight (g)</td>
<td>1.924 ± 0.04</td>
<td>1.937 ± 0.015</td>
<td>1.860 ± 0.02</td>
<td>1.885 ± 0.02</td>
</tr>
</tbody>
</table>

*P at least <0.05 compared with controls; **As compared to WD. Data are mean ± SE.

Table 4. Major brain organic osmolyte contents (μm/g dry brain weight) 24 h after correction of hyponatraemia in rats treated with exogenous urea or water diuresis (WD)

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 10)</th>
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<th>WD (n = 17)</th>
<th>Urea (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myo-inositol</td>
<td>19.3 ± 0.42</td>
<td>10.4 ± 1.3</td>
<td>13.7 ± 0.5*</td>
<td>7.65 ± 0.6**</td>
</tr>
<tr>
<td>Taurine</td>
<td>22.1 ± 0.61</td>
<td>9.04 ± 0.38</td>
<td>13.1 ± 0.68*</td>
<td>8.23 ± 0.32**</td>
</tr>
<tr>
<td>Glutamate</td>
<td>30.2 ± 0.95</td>
<td>30.6 ± 0.73</td>
<td>39 ± 1.6</td>
<td>36.6 ± 1*</td>
</tr>
<tr>
<td>Glutamine</td>
<td>18.9 ± 0.29</td>
<td>9.63 ± 0.44</td>
<td>18.2 ± 1.2</td>
<td>13.9 ± 0.6*</td>
</tr>
<tr>
<td>Creatine</td>
<td>33 ± 0.39</td>
<td>19.3 ± 0.92</td>
<td>54.9 ± 1.1*</td>
<td>33.4 ± 1.1</td>
</tr>
<tr>
<td>Urea</td>
<td>21.7 ± 1.2</td>
<td>6.8 ± 1.1</td>
<td>65.5 ± 7.5*</td>
<td>130 ± 10**</td>
</tr>
<tr>
<td>GABA</td>
<td>10.3 ± 0.31</td>
<td>7.73 ± 0.22</td>
<td>10.6 ± 0.3</td>
<td>10.8 ± 0.32</td>
</tr>
<tr>
<td>Aspartate</td>
<td>6.42 ± 0.15</td>
<td>2.87 ± 0.08</td>
<td>4.4 ± 0.1</td>
<td>4.55 ± 0.14*</td>
</tr>
<tr>
<td>PEA</td>
<td>5.62 ± 0.11</td>
<td>4.81 ± 0.11</td>
<td>5.9 ± 0.3</td>
<td>5.58 ± 0.16</td>
</tr>
<tr>
<td>Betaine</td>
<td>2.39 ± 0.13</td>
<td>1.13 ± 0.06</td>
<td>3.52 ± 0.64</td>
<td>8.56 ± 1.0**</td>
</tr>
<tr>
<td>GPC</td>
<td>1.90 ± 0.10</td>
<td>0.86 ± 0.08</td>
<td>9.72 ± 1.4</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data are mean ± SE. GABA: γ-aminobutyric acid; PEA: Phosphoethanolamine; GPC: glycerophosphorylcholine. *P at least <0.05 compared with controls; **As compared to WD.

Table 5. Minor brain organic osmolyte contents (μm/g dry brain weight) 24 h after correction of hyponatraemia in rats treated with exogenous urea or water diuresis (WD)

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 10)</th>
<th>Hyponatraemia (n = 10)</th>
<th>WD (n = 17)</th>
<th>Urea (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>4.69 ± 0.19</td>
<td>3.59 ± 0.08</td>
<td>7.26 ± 0.3</td>
<td>7.55 ± 0.25*</td>
</tr>
<tr>
<td>Serine</td>
<td>3.93 ± 0.13</td>
<td>2.37 ± 0.07</td>
<td>4.44 ± 0.1</td>
<td>3.27 ± 0.13**</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.24 ± 0.12</td>
<td>1.46 ± 0.05</td>
<td>2.08 ± 0.06</td>
<td>3.02 ± 0.18**</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.87 ± 0.04</td>
<td>1.34 ± 0.03</td>
<td>3.64 ± 0.2</td>
<td>0.05 ± 0.01**</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.75 ± 0.01</td>
<td>2.11 ± 0.06</td>
<td>1.20 ± 0.05</td>
<td>1.12 ± 0.04*</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.53 ± 0.04</td>
<td>0.87 ± 0.09</td>
<td>0.26 ± 0.01</td>
<td>0.33 ± 0.02**</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.29 ± 0.04</td>
<td>0.11 ± 0.01</td>
<td>0.19 ± 0.1</td>
<td>0.14 ± 0.08**</td>
</tr>
<tr>
<td>Valine</td>
<td>0.34 ± 0.01</td>
<td>0.31 ± 0.02</td>
<td>0.42 ± 0.02</td>
<td>0.49 ± 0.02</td>
</tr>
<tr>
<td>Proline</td>
<td>0.33 ± 0.01</td>
<td>0.21 ± 0.01</td>
<td>0.38 ± 0.03</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.22 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.14 ± 0.007</td>
<td>0.29 ± 0.02**</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.22 ± 0.04</td>
<td>0.14 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.33 ± 0.01**</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.10 ± 0.003</td>
<td>0.14 ± 0.01</td>
<td>0.11 ± 0.006</td>
<td>0.13 ± 0.08</td>
</tr>
<tr>
<td>Ornithine</td>
<td>0.07 ± 0.003</td>
<td>0.12 ± 0.01</td>
<td>0.06 ± 0.003</td>
<td>0.052 ± 0.002</td>
</tr>
</tbody>
</table>

*P at least <0.05 compared with controls; **P at least <0.05 compared with water diuresis.
Normal range of the serum sodium in the urea group returned to the control and brain cation contents decreased after hyponatraemia. Serum sodium and plasma data are presented in Table 2. Mean blood urea level was 241 ± 26 mg/dl at 24 h and in the urea group all the rats achieved a blood urea concentration above 100 mg/dl.

**Brain composition.** Brain composition in the Urea group is compared to the brain water electrolyte and osmolyte values of normal controls, chronically (3 days) hyponatraemic rats and following correction with water diuresis.

Brain water and electrolyte contents (Table 3). Normonatraemic rats (*n* = 10) are used as controls for brain composition. In hyponatraemic rats (*n* = 16), brain water content was increased compared to controls and brain cation contents decreased [9].

Brain water contents measured 24 h after correction of the serum sodium in the urea group returned to the normal range [3.42 ± 0.021 H2O/kg dry brain weight (DBW)]. In contrast, brain water in the group treated by water diuresis was significantly increased compared to controls and urea group (3.55 ± 0.021 H2O/kg DBW).

Of note, there was no brain Na overshoot in the urea group compared to animals corrected with water diuresis (brain Na: 202 ± 2.5 in urea treated vs 219 ± 3.1 in water diuresis group, *P* < 0.05 controls: 192 ± 1.8 μEq/g DBW) (Table 3).

Brain potassium content was significantly lower in the urea-treated group compared to the group corrected with water diuresis or to controls.

Brain organic osmolytes (Tables 4 and 5). Table 4 shows the data of the major brain organic osmolyte contents in controls, after hyponatraemia (3 days) and 24 h after correction of the hyponatraemia with both urea and water diuresis. In hyponatraemia, major brain organic osmolyte contents decreased as expected [9].

Table 5 presents the data of minor (brain content <5 μM/g DBW) organic osmolytes.

As expected, the urea-treated group shows a significant increase in brain urea contents compared to controls and to the water diuresis group.

The only significant change (Table 4) obtained in the organic osmolyte profile of urea-treated animals include the increase in brain betaine contents compared to both the control group (a 4-fold increase) and to the water diuresis group.

The levels of the other trimethylamines (GPC and PEA) were normalized at the time of sodium correction after urea treatment (GPC is lacking), but increased significantly above the normal values for GPC in the water diuresis group.

Brains of the urea-treated animals remained depleted in myo-inositol and taurine at 24 h. Their levels were significantly lower than in the water diuresis group.

Glutamate increased only slightly in the urea group. Glutamine was partly corrected after urea, but was normalized in the water diuresis group.

Among the ‘minor’ brain amino acids, only alanine, tyrosine and isoleucine levels remained decreased in the urea group compared to the control values (Table 5).

The other amino-acids were normalized or increased above the control levels at 24 h. We also analysed separately the data coming from rats with blood urea levels at 24 hr <150 mg/dl to evaluate its potential influence on the brain response to the correction of the serum sodium.

In the group with blood urea above 150 mg/dl (mean urea: 311 ± 31 mg/dl, range 158–581, *n* = 16) the brain water, electrolyte and organic osmolyte contents were comparable to the data coming from the lower blood urea level’s group (mean urea value 128 ± 13 mg/dl, range 111–142, *n* = 10).

**Discussion**

Neurological damage complicating correction of chronic hyponatraemia is responsible for substantial morbidity and mortality [1,4].

Since the first experimental demonstrations [1] of the relationship between correction of hyponatraemia and development of demyelinating brain lesions, many cases of ODS were reported and continue to be published despite relative consensus on the approach to treatment [13,14]. The exact incidence of brain demyelination after correction of severe (SNa <115 mEq/l), chronic (>48 h) hyponatraemia is unknown, but ranges probably between 5 to 20% in unselected cases [15].

Patients develop ODS because of excessive correction of hyponatraemia with sometimes unpredictable brisk increase in serum sodium or because of unrecognized associated risk factors [1,4]. Sometimes also, apparently, slow gradient of correction leads to ODS because of a possible rise in serum sodium prior to hospital admission. The true osmotic gradient sustained by the patient is then underestimated by the clinician.

There is a need for safer therapeutic options to treat hyponatraemia. Treatment of hyponatraemia with urea has been proposed for more than 20 years [3]. It is widely used in our hospitals without recognizable neurological complications [1,4].

Previous experimental data also suggested that urea may protect against ODS [2]. In this initial work, we showed that both the incidence (89% to 39% vs hypertonic saline) and the severity of brain lesions were significantly reduced when urea was used to treat chronic hyponatraemic rats [1,2]. In this preliminary work [2], however, a relatively low (50–120 mg/dl) blood level of urea was achieved. In addition, the levels were not sustained: urea returned to almost normal range before the next i.p. injection.

The effect of higher and more sustained levels was determined in the present study.

Repeated infusions of urea during 24 h maintained plasma levels between 150 and 250 mg/dl and the lower levels remained above 60 mg/dl.
Prevention of myelinolysis after correction of hyponatraemia with urea

A large gradient of serum sodium (≈32 mEq/l/24 h) known to generally induce high morbidity and mortality rates was well tolerated by the rats in this group. Indeed, the outcome in animals treated with urea was largely favourable with only 13% mortality.

With similar correction levels in the group treated with AVP withdrawal, the outcome was catastrophic. Mortality rate was high and almost all rats developed neurological manifestations related to myelinolysis. Different therapeutic modalities have been evaluated for chronic hyponatraemia in animals, but whatever the treatment used including water diuresis, hypertonic saline or vasopressin receptor antagonists [16–18], morbidity and mortality rates were high (±80–90%) for correction levels >25 mEq/l/24 h [1]. Future treatment of hyponatraemia with vasopressin-receptor antagonists [19], like other therapies, is also theoretically at risk for myelinolysis in human. Only urea has been demonstrated to be almost safe at high correction rates. Given the present results and previous data, urea provides protective effects for brain exposed to major osmotic stress during hyponatraemia.

In the second part of the present work, in an attempt to explain the mechanism of the protective effects of urea, we analysed the brain composition in response to an abrupt correction of serum sodium with urea. Brain composition of hyponatraemic rats corrected by water diuresis was also determined, a model which induced comparable incidence of brain damage to correction with hypertonic saline [8,9]. We also compared these results to the data obtained in our previous azotemic model [8]. Azotemic (48 h) brain was shown to be resistant to the development of myelinolysis [8] and we demonstrate that it was able to rapidly reaccumulate its organic osmolyte contents [9]. Myo-inositol, taurine, glutamine and betaine which represent the major non-perturbing osmolytes were normalized in azotemic rats after 24 h of correction. In non-azotemic environment, this reuptake takes 5–7 days to complete [6].

We have interpreted the resistance of uraemic brain against ODS in our azotemic model as the consequence of its ability to quickly (2–24 h) re-establish its intracerebral organic osmolyte contents. The mechanism by which azotemic brain normalized rapidly its osmolyte contents is unknown. It is possible that in renal failure, the osmolytes accumulate in blood and become immediately available for the brain in case of osmotic stress. This rapid reuptake of the organic osmolytes could prevent subsequent excessive brain dehydration and brain hyperionization (NaCl) responsible for further development of the demyelinating processes and neuronal death. This sequence of events represents the classical scenario proposed in ODS [1,6]. Obviously, this observation does not exclude involvement of a specific protective effect of urea itself to explain the favourable influence exerted by the azotemic milieu on brain [8].

Consistent with this possibility, brain analysis obtained 24 h after correction of hyponatraemia with exogenous urea did not reproduce the brain adaptative response observed in our previous model with renal failure. Despite a similar gradient of correction and comparable favourable outcome [8], the brain contents of major organic osmolytes (myo-inositol, taurine, glutamine, aspartate) remained decreased 24 h after correction of the serum sodium with urea (Table 4). Betaine levels alone were increased.

Also, as expected, brain urea contents were increased in the urea-treated group. However, its concentration was half of that observed in the azotemic model (130 ± 10 vs 289 ± 4 μm/g DBW [9], paralleling the serum urea values. Unlike these differences between azotemic and urea-treated animals in the brain organic osmolyte composition, cerebral water contents were similar in both models [9]). In addition, brain NaCl overshoot was also prevented by urea (Table 3) as well as in azotemia [9]. This brain hyperionization developed after treatment of hyponatraemia with hypertonic saline or water diuresis [6,9] (Table 3) and is potentially implicated in the genesis of myelinolysis [1].

The brain remained partially depleted in potassium in rats treated with urea (Table 3). We have no satisfactory explanation for this, but it is possible that a gradient of urea between blood and brain exists (because of repeated administrations of urea, compared to more constant values in the previous azotemic model) and that a higher brain urea content, even temporarily, slows the brain K+ reaccumulation.

In our control group with high incidence of brain damage treated by water diuresis, as expected, the brain remained depleted in the major organic osmolyte contents (myo-inositol and taurine), although somewhat less than in the urea group.

The fact that a similar level of brain protection was observed in azotemic [9] and urea-treated animals with such differences in brain osmolyte composition is intriguing. Accumulation of organic osmolytes in the blood of azotemic rats (renal failure) could be a potential mechanism to explain why the reuptake of brain organic osmolytes was rapid (24 h) in this model and was not observed in rats treated with exogenous urea. The common factor between both models (azotemia and rats treated with urea), which lacks in the other correction methods (water diuresis, hypertonic saline) also associated with the higher incidence of brain damage, is the increased blood and cerebral urea concentrations. Our present observation confirms the favourable influence of an urea-enriched milieu and strengthens our hypothesis for the contribution of specific properties of urea itself in the protective mechanisms against myelinolysis [8,9].

Urea administration was associated with the rapid (24 h) brain reuptake of only betaine, one of the major osmoprotective osmolytes, which increased above normal levels.

It is possible that organic osmolytes are interchangeable during the cells’ response to osmotic stress. Then, the lack of accumulation of some organic osmolytes could be compensated for by the increase of another one in order to prevent further cell shrinkage and death. Protective effects of betaine
against hypertonic stress were recently shown in vivo in rats and in cultured kidney (MDCK) cells [20]. In the future, exogenous administration of myo-inositol [21] and other major osmoprotective osmolytes during correction of hyponatraemia may be an effective approach to prevent brain demyelination.

Recent data suggest that apoptosis participates in the pathogenesis of central pontine myelinolysis (and ODS), probably as a consequence of the glial cells’ metabolic modifications arising during adaptation to changes in osmolality [22].

A cascade of events has been implicated in the genesis of ODS. Other mechanisms are suspected to be involved in ODS like intracerebral hyperosmolarization (NaCl) [1], blood–brain-barrier disruption allowing influx of myelino-toxic factors and complement activation into the brain [23].

There are additional possible mechanisms for the effectiveness of urea.

Prevention of excessive brain dehydration and neurological damage via brain urea and water retention as observed in the ‘dialysis disequilibrium syndrome’ [24] is very unlikely in both our models (azotemia and urea-treated animals).

In uraemia, equilibration of urea between plasma and brain is slow (4–12 h) as a consequence of massive reduction of urea brain transporters (UTB1) [1,25]. However, in our experimental models, plasma urea levels continued to rise during correction of hyponatraemia, promoting rather than counteracting the dehydrating effect on the brain of the increase in serum sodium.

A more likely possibility is the role of urea as a protective osmolyte. Organisms living in osmotically challenging environments accumulate osmolytes in their body fluids to maintain osmotic equilibrium.

In elasmobranchs that have thrived for millions of years in sea water, the main low-molecular weight substance is represented by urea in high concentration (400 mM) balancing the osmolality of the external milieu (sea water NaCl 25 g/l ≈1000 mOsm/kg H2O). However, the body fluid composition of sharks shows also high plasma sodium (200–280 mEq/l) and potassium (7–14 mEq/l) concentrations to equilibrate their osmolality (total plasma osmolality in sharks ≈1000 mOsm/kg H2O).

Living with high plasma salt concentration in these species is probably possible only because of their urea-retaining system, urea counteracting the toxic effects of NaCl.

This story of the species evolution may have relevance to mammalian physiology through our observation that urea protects brain cells against ODS in our experimental models. Another organ in which internal environment has a strong similarity with that of elasmobranchs is the renal medulla. Interestingly, a recent work in renal medullary cultured cells showed that urea pretreatment protected cells from the proapoptotic effect of hypertonic stress induced by NaCl [26]. This effect was dose-dependant and may be also implicated in brain protection.

Striking protective effects of high urea levels against oxidative stress were demonstrated in shark hearts and then reproduced in the hearts of rats at a concentration-dependant effect [27]. Urea may also be a brain protective agent through these spectacular antioxidative properties observed in the heart.

In conclusion, we have confirmed the strongly protective effect of urea against ODS and showed that the adaptative response of urea-treated brains differs from the azotemic model [9], suggesting the existence of more specific protective mechanisms for urea.

In clinical practice, current recommendations, for treatments of chronic hyponatraemia, are to limit the correction by <10 mEq/l/24 h to avoid the risk of developing brain myelinolysis [4]. However, a large increase in serum sodium could be unpredictable and treatment of hyponatraemia with urea could avoid disastrous neurological sequelae in these patients.

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