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CLINICAL INVESTIGATION

Depletion of glutathione from brain cells in hyponatremia

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Depletion of glutathione from brain cells in hyponatremia. In response to hyponatremia, brain cells extrude electrolytes and organic osmolytes, thereby minimizing brain edema. We demonstrate that rat brain is depleted of the antioxidant glutathione in response to hyponatremia and that osmotically-induced loss of glutathione makes neuronal cells more susceptible to oxidative injury. Total glutathione content of brain tissue decreased from 6.80 ± 0.14 μmol/g dry wt in normonatremic controls to 5.00 ± 0.31 μmol/g dry wt after 72 hours of hyponatremia. Following slow correction of hyponatremia, brain glutathione content returned to control values (6.77 ± 0.34 μmol/g dry wt). Brain content of taurine, a β-amino acid with antioxidant properties, similarly decreased in hyponatremia (29.6 ± 0.9 to 17.1 ± 1.2 μmol/g dry wt), then increased with slow correction (24.8 ± 1.3 μmol/g dry wt). Although taurine served as an osmolyte in rat heart, liver and brain, osmotically-induced changes in glutathione content were found only in brain. We also studied osmotically-induced changes in glutathione and taurine content in C6 glioma and SK-N-SH neuroblastoma cells. In both cell lines, adaptive decreases in glutathione and taurine content were found in response to lowering medium sodium concentration from 140 mM to 100 mM. The cell content of these solutes increased after returning to media containing 140 mM sodium. Following exposure of both cell lines to hypoosmolar media, there was no increase in media content of glutathione. This suggests that osmotic depletion of glutathione is not due to cellular efflux of intact glutathione. We questioned if osmotic depletion of glutathione and taurine renders brain cells more susceptible to oxidative stress. Incubation of SK-N-SH cells with 1.0 mM H_2O_2 for four hours induced greater cytolytic injury in cells adapted to hypoosmolar media than in isoosmolar controls. Hypoosmolar C6 glioma cells were not significantly more sensitive to cytolytic injury from H_2O_2 than were cells grown in isosmolar media. We conclude that hypoosmolality induces glutathione depletion in rat brain *in vivo* and in cultured brain cells *in vitro*. Osmotic depletion of this antioxidant renders SK-N-SH neuronal cells more susceptible to oxidative injury.

In hyponatremia, reduction of brain solute content minimizes osmotically-induced brain swelling. These solutes include electrolytes and organic osmolytes such as amino acids, polyols, and trimethylamines [1, 2]. Maintenance of constant water content in the face of extracellular hypoosmolality assumes particular importance in brain given the rigid volume constraints imposed by the surrounding calvarium. Loss of intracellular solutes in response to reduced extracellular fluid osmolality has also been identified in cultured brain cells *in vitro* [3].

Prominent among the organic osmolytes extruded from brain cells in hyponatremia is the β-amino acid, taurine [1]. In a variety of *in vitro* and *in vivo* models of oxidative injury, taurine, which is normally present in brain at a concentration of approximately 5 mM [4], has been shown to serve as an antioxidant [5–9]. Taurine also ameliorates hypoxic brain injury [10–13]. The tripeptide glutathione, the most abundant intracellular thiol in rat brain (normally present at a concentration of approximately 2 mM) [14] is well established as an important antioxidant. We demonstrate that rat brain *in vivo* and cultured brain cells *in vitro* undergo adaptive decreases in glutathione content in response to a reduction in extracellular fluid osmolality. We hypothesized that adaptive decreases in the cell content of the antioxidants taurine and glutathione in response to extracellular hypoosmolality renders brain cells more susceptible to superimposed oxidative injury. This hypothesis was tested in cultured brain cells adapted to hypoosmolar medium.

Methods

In vivo studies

Brain glutathione and taurine content were determined in three groups of male Sprague-Dawley rats (300 to 350 g body wt): normonatremic controls, chronic hyponatremia, and slowly corrected chronic hyponatremia. Chronic hyponatremia was induced by the subcutaneous infusion of desmopressin acetate (DDAVP, Rhone-Poulenc Rorer, Malmo, Sweden) by mini-osmotic pump (Alzet, Palo Alto, CA, USA) at a rate of 20 ng/hr for 72 hours. All animals were gavage fed a liquid low-electrolyte chow (ICN Biomedicals, Cleveland, OH, USA) providing 9 ml H_2O/100 g body wt daily over the three-day induction period. Normonatremic control animals were treated and fed identically to hyponatremic rats except that mini-osmotic pumps contained only normal saline. All animals were housed with a 12-hour light-dark cycle and allowed free access to water overnight. Slow correction was achieved by changing three-day hyponatremic rats from low-electrolyte chow to regular chow for seven days and allowing the mini-osmotic pumps to exhaust their desmopressin content. All rats appeared well throughout the study period and survived until sacrificed.

Normonatremic controls and chronic hyponatremia rats were sacrificed for analysis at 72 hours and slowly corrected animals at 10 days. Animals were sacrificed by guillotine and their skulls immediately bisected sagittally with an electric table saw. Both hemispheres were promptly removed with one being placed in liquid nitrogen for determination of glutathione and taurine content, and the other hemisphere analyzed for water and electrolyte content. No more than 15 seconds elapsed from the time of sacrifice to the placement of brain tissue in liquid nitrogen. The heart and liver were also rapidly removed and frozen. Frozen

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tissue was crushed under liquid nitrogen and total glutathione and taurine were measured by reverse-phase HPLC following derivitization with phenylisothiocyanate (Pico-Tag, Waters, Milford, MA, USA). Methionine sulfone was added to each tissue sample as an internal standard. During derivitization, a significant fraction of reduced glutathione (GSH) is oxidized to glutathione disulfide (GSSG). Total glutathione was therefore calculated as the sum of the GSH and GSSG peaks from each chromatogram. Initial studies verified greater than 98% recovery of exogenous GSH added to tissue samples. Total glutathione is expressed as GSH equivalents.

Our technique of harvesting brain tissue was employed because it allows the removal of both hemispheres intact and avoids the need for anesthetic agents. Because there likely is some degree of oxidation of GSH to GSSG during harvesting, we measured total glutathione (GSH and GSSG). This approach has been employed previously in studies of brain glutathione metabolism where brain tissue was similarly harvested immediately after decapitation [15, 16]. We did not measure brain lactate as part of our analysis.

Immediately upon removal, the remaining brain hemisphere was weighed, placed in an oven at 100°C for 48 hours, and then reweighed to determine its water content. Dried brain was then crushed and extracted in 0.75 N HNO_3 for 72 hours. Sodium and potassium contents were determined by flame photometry (Instrumentation Laboratories, Model 943, Lexington, MA, USA). Brain water and solute data are expressed per gram of tissue dry weight. Plasma sodium was determined by flame photometry on trunk blood obtained at the time of sacrifice.

In vitro studies

C6 rat glioma cells and SK-N-SH human neuroblastoma cells were obtained from American Type Culture Collection (Bethesda, MD, USA). C6 cells are a rat glial cell line which possess several biochemical characteristics of oligodendroglia such as high levels of 2′3′ cyclic nucleotide, 3′ phosphorylase and myelin components [17, 18]. SK-N-SH cells are a human neuroblastoma cell line with biochemical markers indicating neuronal origin, such as the presence of dopamine β-hydroxylase and both α-2 adrenergic and cholinergic receptors [19, 20]. The cells were grown to confluence in Modified Eagle's Medium (MEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (MEM/FBS, Sigma Chemical Company, St. Louis, MO, USA) in multiwell culture plates in a humidified incubator at 37°C with 5% CO_2.

Once confluence was reached (3 days for C6 cells and 7 to 10 days for SK-N-SH cells), medium osmolality was lowered by replacing existing media every 12 hours with media having a sodium concentration that was 10 mM lower. Thus, the medium sodium concentration was lowered from 140 mM to 100 mM over 48 hours. Media with sodium concentrations of 140 mM, 130 mM, 120 mM, 110 mM and 100 mM were prepared to insure that their substrate concentrations were identical. Stock MEM was diluted with sterile water such that when 10% fetal calf serum was added, a final sodium concentration of 100 mM was achieved. NaCl was then added to aliquots of this media to achieve media of graded sodium concentrations. Potassium concentration in all media was adjusted to 5 mM. Based on preliminary studies, bicarbonate concentration in all media was adjusted to 26 mM in order to maintain a measured pH of approximately 7.40 to 7.45 at the termination of each study protocol. In wells where medium osmolality was corrected back to normal, media was again changed every 12 hours, increasing the sodium concentration in 10 mM increments from 100 mM to 140 mM over 48 hours. Cell viability was >98% in representative wells by Trypan blue exclusion.

Total glutathione and taurine content in C6 and SK-N-SH cells were determined under three conditions: isosmolar, hypoosmolar, and hypoosmolar corrected to isosmolar. In this protocol, isosmolar cells were maintained in a medium with a sodium concentration of 140 mM for 96 hours, while in hypoosmolar cells sodium concentration was lowered from 140 mM to 100 mM over 48 hours and then kept at 100 mM for an additional 48 hours. For corrected hypoosmolality, the sodium concentration was lowered from 140 mM to 100 mM over 48 hours and subsequently increased back to 140 mM over an additional 48 hours. In all wells, media was replaced every 12 hours with media of appropriate sodium concentration.

At the termination of this protocol, media was aspirated from each well and the cell monolayer washed twice with Hank's buffered salt solution (HBSS) of appropriate osmolality. Cell monolayers were then lysed with 0.1 N HCl, derivitized with phenylisothiocyanate, and total glutathione and taurine concentrations were determined by HPLC. For each well, methionine sulfone was added as an internal standard. Solute data are expressed per milligram cell protein.

To explore the possibility of cellular efflux of glutathione as the mechanism underlying its depletion by hypoosmolality, the following studies were performed. Both cell lines were grown to confluence in six well-culture plates with isosmotic MEM/FBS. MEM/FBS was then aspirated and each well washed three times with isosmotic HBSS. A total of 1.5 ml MEM without FBS but with sodium concentration adjusted to either 100 mM or 140 mM was then added to culture wells. After a 60-minute incubation, media were aspirated and solute content determined by HPLC as described above. A 60-minute incubation period was chosen based on the studies of Yudkoff and co-workers, who measured the efflux of glutathione from cultured astrocytes [21]. Stock MEM contains no glutathione or taurine. Because of the large media volume relative to cellular volume, serum-free media was used in these studies to ensure that any measured glutathione or taurine in the media was due to cellular efflux. There was no detectable glutathione or taurine in serum-free MEM before incubation with brain cells when analyzed by our HPLC methodology. Quantities of glutathione and taurine measured in the media after incubation with brain cells were well within the sensitivity of our HPLC analysis. Results are expressed as total nmoles in the media of each well per milligram cell protein. Cell protein content was measured by the Lowry method [22].

In separate studies, cultured brain cells adapted to hypoosmolar medium were exposed to hydrogen peroxide to study their susceptibility to oxidative stress. Because of the ability of α-keto acids to efficiently scavenge H_2O_2 via non-enzymatic oxidative decarboxylation [23], all culture media were pyruvate-free. SK-N-SH cells were grown to confluence and medium sodium concentration lowered from 140 mM to 100 mM over 48 hours as described above. In control cells, sodium concentration was maintained at 140 mM. Cytolytic injury to cultured brain cells was determined by ^{51}Cr release as previously described [23]. To load cells with ^{51}Cr, media in each well was aspirated and replaced with 1 ml of HBSS of appropriate osmolality containing 1 μCi sodium

Table 1. Plasma sodium, brain water and brain electrolyte content in chronic hyponatremia

	Control	Hyponatremia	Corrected hyponatremia
Plasma Na^+ *mM*	143 ± 1	108 ± 3[a]	140 ± 1
Brain H_2O *ml/g dry wt*	3.84 ± 0.02	4.01 ± 0.03[b]	3.95 ± 0.05
Brain K^+ *μEq/g dry wt*	487 ± 5	434 ± 8[a]	515 ± 4[b]
Brain Na^+ *μEq/g dry wt*	228 ± 2	196 ± 4[a]	247 ± 8

[a] $P < 0.05$ vs. control and corrected hyponatremia
[b] $P < 0.05$ vs. control, $N = 9$ for all groups

Table 2. Tissue glutathione and taurine content in chronic hyponatremia

	Control	Hyponatremia	Corrected hyponatremia
Brain			
Glutathione	6.80 ± 0.14	5.00 ± 0.31[a]	6.77 ± 0.36
Taurine	29.6 ± 0.9	17.1 ± 1.2[a]	24.8 ± 1.3[c]
N	9	9	9
Heart			
Glutathione	4.96 ± 0.46	5.01 ± 0.29	5.01 ± 0.34
Taurine	82.6 ± 6.5	63.5 ± 4.6[b]	80.8 ± 5.4
N	9	9	9
Liver			
Glutathione	24.77 ± 2.44	26.37 ± 1.78	21.97 ± 2.16
Taurine	13.9 ± 2.9	3.8 ± 0.5[a]	10.4 ± 1.1
N	9	8	8

Data are expressed as μmol/g dry wt.
[a] $P < 0.01$ vs. control and corrected
[b] $P < 0.05$ vs. control and corrected
[c] $P < 0.05$ vs. control

[^{51}Cr]chromate (Amersham Corp., Arlington Heights, IL, USA). Following a three-hour incubation with sodium [^{51}Cr] chromate, cells were washed twice with HBSS, then media of appropriate osmolality was replaced. After ^{51}Cr loading, isosmolar and hypoosmolar SK-N-SH cells were incubated with 1.0 mM H_2O_2 for four hours at 37°C with 5% CO_2. Following this four hour incubation, media was aspirated, combined with two washes with HBSS of appropriate osmolality, and ^{51}Cr activity counted. The remaining cell monolayer was solubilized with 2 ml of 1.0 N NaOH, aspirated, and combined with two 2-ml washes of the well with water and counted separately. Percent ^{51}Cr release was calculated as ^{51}Cr activity in the overlying medium divided by total ^{51}Cr activity. Cytolytic injury of isosmolar and hypoosmolar C6 glioma cells was similarly studied after a four hour incubation with 1.0 mM H_2O_2.

Statistical analysis

Data are presented as means ± standard error. In cell culture studies, *N* refers to the number of replicate wells in each experimental condition. The unpaired Student's *t*-test was utilized to analyze differences between studies with two groups. In studies of three or more groups, the unpaired Student's *t*-test with Bonferroni's correction was employed. A *P* value <0.05 was considered statistically significant.

Results

Tissue glutathione and taurine content in hyponatremia

Hyponatremia induced over three days caused the plasma sodium to decrease from 143 ± 1 mM in controls to 108 ± 3 mM in the hyponatremic group (Table 1). Following slow correction of hyponatremia, plasma sodium increased to 140 ± 1 mM. Brain water increased significantly from 3.84 ± 0.02 ml/g dry wt in controls to 4.01 ± 0.03 ml/g dry wt in hyponatremia and returned toward control values following the slow correction of hyponatremia. Brain sodium and potassium contents decreased significantly in hyponatremia and increased following its slow correction.

Brain total glutathione content decreased significantly in response to hyponatremia, falling from 6.80 ± 0.14 to 5.00 ± 0.31 μmol/g dry wt (Table 2). Brain taurine content similarly declined from 29.6 ± 0.9 μmol/g dry wt in controls to 17.1 ± 1.2 μmol/g dry wt in the hyponatremic group. Both glutathione and taurine returned to levels close to control following the correction of hyponatremia. The concentration of total glutathione in brain water can be calculated by dividing the brain glutathione content (μmol/g dry wt) in each rat by its respective brain water content (ml/g dry wt). In control, hyponatremic and corrected groups, this yields values of 1.77 ± 0.64 mM, 1.25 ± 0.07 mM, and 1.72 ± 0.10 mM, respectively. Corresponding values for brain taurine concentration are 7.71 ± 0.22 mM, 4.26 ± 0.30 mM, and 6.28 ± 0.52 mM.

In heart tissue, taurine content decreased in response to hyponatremia and returned to control values following its correction (Table 2). Total glutathione content, however, remained constant in heart tissue in all three experimental conditions. A similar pattern was identified in liver tissue with taurine content decreasing in the hyponatremic group and returning toward control values in slowly corrected rats. There was no statistical difference in liver glutathione content between any of the three groups. Thus, while taurine functioned as an organic osmolyte in all three tissues studied, osmotic depletion of glutathione was found only in brain. In Figure 1, individual data from all three groups are pooled and tissue total glutathione and taurine contents are plotted against plasma sodium. Brain total glutathione correlates significantly with plasma sodium ($r = 0.79$, $P < 0.01$), further indicating the osmotic responsiveness of glutathione in brain; however, no such correlation was found for heart and liver. Taurine content correlated significantly with plasma sodium in all three tissues.

Glutathione and taurine content in cultured brain cells

In both C6 glioma and SK-N-SH neuroblastoma cells, hypotonicity-induced depletion of both glutathione and taurine was identified (Table 3). Following the reduction in medium sodium concentration from 140 mM to 100 mM, glutathione content in C6 cells fell from 6.33 ± 1.94 to 4.26 ± 1.04 nmol/mg protein. Taurine content fell dramatically from 67.1 ± 3.7 nmol/mg protein to undetectable levels. Cell content of both glutathione and taurine increased significantly from hypoosmolar values following the return to an isotonic culture medium. In SK-N-SH cells, similar findings were noted with glutathione and taurine contents decreasing significantly in response to hypoosmolality and returning toward control values with correction of medium osmolality (Table 3). Glutathione decreased from 4.33 ± 0.98 nmol/mg protein in isosmolar control cells to 2.01 ± 0.13 nmol/mg protein in hypoosmolar cells and subsequently increased to 4.38 ± 0.65

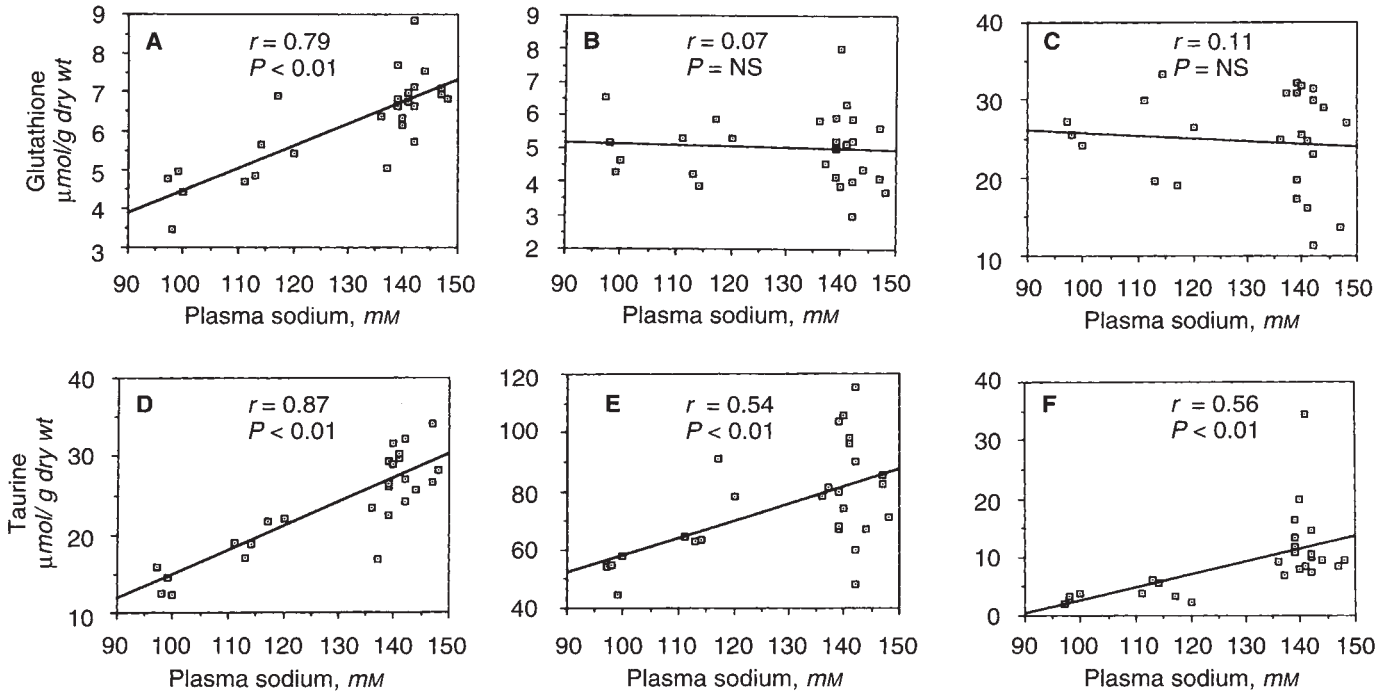

Fig. 1. *Correlation of total glutathione content in brain (A), heart (B), liver (C) and taurine content in brain (D), heart (E) and liver (F) with plasma sodium in chronically hyponatremic, corrected hyponatremia, and normonatremic control rats.* Data are pooled from all rats presented in Tables 1 and 2. $N = 27$ for brain and heart and 25 for liver.

Table 3. Glutathione and taurine content in cultured brain cells

	Medium Na^+ concentration		
	140 mM	100 mM	100 mM corrected to 140 mM
C6 glioma cells			
Glutathione	6.33 ± 1.94	4.26 ± 1.04[a]	11.88 ± 2.62[b]
Taurine	67.1 ± 3.7	ND[a]	32.5 ± 2.1[b]
N	6	5	5
SK-N-SH neuroblastoma cells			
Glutathione	4.33 ± 0.98	2.01 ± 0.13[a]	4.38 ± 0.65
Taurine	39.3 ± 2.4	4.8 ± 0.1[a]	34.1 ± 0.9
N	6	7	7

Data are expressed as nmol/mg protein, n indicates the number of replicate wells in each group. Abbreviation ND is none detected.
[a] $P < 0.01$ vs. 140 mM and corrected groups
[b] $P < 0.01$ vs. 140 mM

Table 4. Culture media content of glutathione and taurine following 60-minute exposure to hypoosmolar MEM

MEM sodium (mM)	C6 glioma		SK-N-SH neuroblastoma	
	140	100	140	100
GSH	3.31 ± 0.40	3.57 ± 0.26	1.72 ± 0.25	1.96 ± .53
Taurine	9.8 ± 2.0	36.0 ± 1.1	19.4 ± 3.5	20.8 ± 2.4

Data are expressed as nmol/mg cell protein. $N = 6$ for each group.
[a] $P < 0.001$ vs. 140 mM

nmol/mg protein in corrected cells. Respective values for taurine were 39.3 ± 2.4, 4.8 ± 0.1, and 34.1 ± 0.9 nmol/mg protein.

Cellular efflux of glutathione and taurine

Following exposure of both cell lines to either isosmolar or hypoosmolar media, there was no difference in the media content of glutathione (Table 4). As has previously been described for glial cells [3], we identified significant efflux of taurine from C6 glioma cells in response to hypoosmolality.

Oxidative injury in cultured brain cells

Release of ^{51}Cr from hypoosmolar SK-N-SH cells exposed to 1.0 mM H_2O_2 was significantly greater than that in isosmolar cells (35.4 ± 1.7% vs. 19.0 ± 2.4%; Fig. 2A; $P < 0.001$). H_2O_2-induced cytolytic injury in hypoosmolar C6 glioma cells did not differ significantly from injury in C6 cells grown in isosmolar media (Fig. 2B). ^{51}Cr release from SK-N-SH cells in both isosmolar and hypoosmolar media was significantly greater than the respective ^{51}Cr release from C6 cells ($P < 0.05$, Fig. 2 A, B). In preliminary studies, hypoosmolality alone did not increase ^{51}Cr release above isosmolar background levels from both SK-N-SH and C6 cells.

Discussion

Solute loss in response to extracellular hypoosmolality is a beneficial adaptation in brain cells because it reduces brain edema and avoids life-threatening increases in intracranial pressure. However, there may be adverse consequences to osmotically-induced depletion of metabolically important solutes. We present *in vivo* and *in vitro* data demonstrating depletion of the antioxidants glutathione and taurine from rat brain and cultured brain cells in response to hypoosmolality.

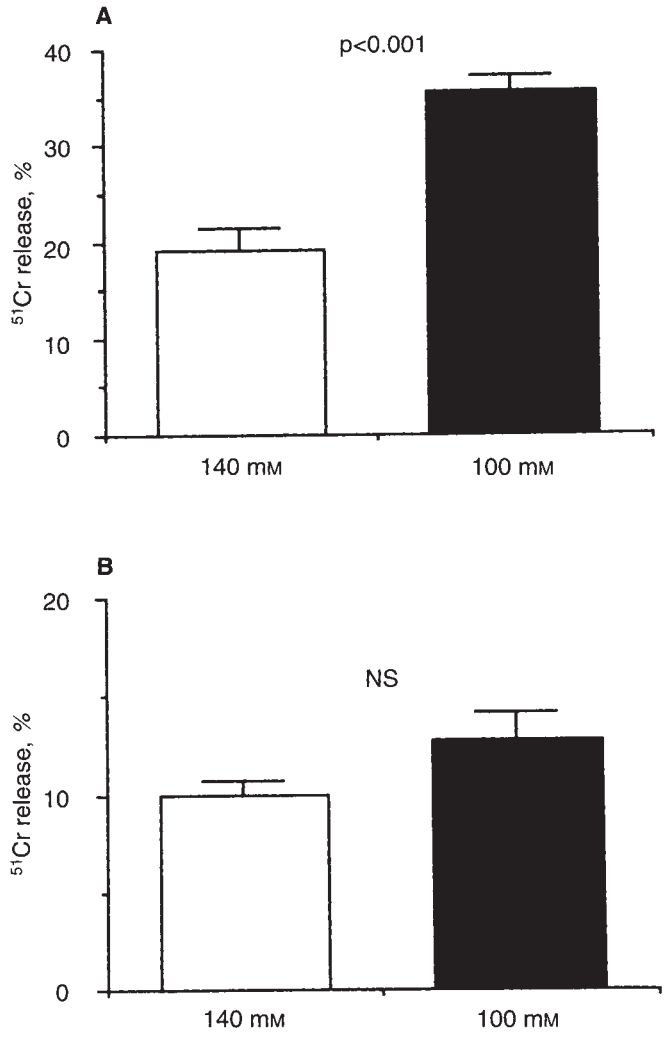

Fig. 2. A. *Effect of hypoosmolality on H_2O_2-induced cytolytic injury in SK-N-SH cells.* $N = 12$ for both experimental conditions. Hypoosmolar SK-N-SH cells were incubated in media of progressively lower sodium concentration over 48 hours with final sodium concentration of 100 mM (■). Controls were maintained in isosmotic medium with sodium concentration of 140 mM (□). Cytolytic injury was quantitated with ^{51}Cr release after a 4-hour incubation with 1.0 mM H_2O_2. ^{51}Cr release in hypoosmolar group is significantly greater than in isosmolar group ($P < 0.001$). ^{51}Cr release from SK-N-SH cells in both isosmotic and hypoosmotic conditions is significantly greater than the respective values below for C6 glioma cells ($P < 0.05$). **B.** Effect of hypoosmolality on cytolytic injury induced by 1.0 mM H_2O_2 in C6 glioma cells. $N = 6$ for both groups. C6 glioma cells were incubated in media of progressively lower sodium concentration over 48 hours with final sodium concentration of 100 mM (■). Controls were maintained in isosmotic medium with sodium concentration of 140 mM (□). Cytolytic injury was quantitated with ^{51}Cr release after a 4-hour incubation with 1.0 mM H_2O_2. ns - no significant difference between 100 mM and 140 mM.

Brain total glutathione and taurine content decreased in response to three days of hyponatremia and subsequently increased following slow normalization of plasma sodium. Brain glutathione correlated closely with plasma sodium across a wide range of values. This osmotic sensitivity of glutathione was not found in heart or liver, a finding which may reflect the more rigid volume constraints placed on the brain compared to other tissues. We further studied the adaptation to hypoosmolality in cell lines of glial and neuronal origin. In C6 glioma and SK-N-SH neuroblastoma cells, contents of glutathione and taurine were significantly reduced in response to a reduction in extracellular osmolality.

The specific cellular mechanisms responsible for these adaptive changes in glutathione content are unknown. Glutathione is both synthesized in brain from constituent amino acids [16, 24] and transported directly across the blood-brain barrier as an intact tripeptide [25]. In studies utilizing cultured astrocytes in isosmotic media, Yudkoff and co-workers demonstrated release of intact glutathione from these cells into the culture medium [21]. Medium concentration of glutathione in these studies reached a steady state by 60 minutes following the change of media. We therefore examined glutathione efflux from C6 glioma and SK-N-SH neuroblastoma cells following a 60-minute exposure to hypoosmolar culture media. For both cell types, we found no differences in the media content of glutathione in hypoosmolar media when compared to isosmolar controls. This finding suggests that osmotic depletion of brain cell glutathione is more likely due to diminished synthesis. Given the rapid turnover of glutathione in brain cells [21], a decrease in synthetic rate could quickly translate into reduced cell content of glutathione. Significant efflux of taurine from C6 cells into hypoosmolar media was identified and is consistent with previous reports of osmotically-induced taurine loss from astrocytes [3]. Osmotically-induced release of taurine from cultured astrocytes occurs via pathways sensitive to anion channel inhibitors [3]. Activation of these pathways is the most likely explanation for these adaptive changes in cell taurine content. Uptake of taurine by brain cells in response to hyperosmolality appears to occur via a different transport mechanism [26, 27].

The importance of glutathione as an antioxidant in brain is well established. Depletion of glutathione to levels approximately two-thirds of control values by buthionine sulfoximine makes rat brain more susceptible to focal ischemic injury induced by middle cerebral artery ligation [16]. This degree of glutathione depletion approximates that found in the present studies of hyponatremia. Similarly, global brain injury imposed by hyperbaric hyperoxia is exacerbated by diethylmaleate-induced glutathione depletion [15]. In addition to the role of glutathione in scavenging H_2O_2 and organic peroxides, Yudkoff and co-workers raise the intriguing possibility that glutathione synthesis by glial cells may serve as a disposal pathway for glutamate, thereby minimizing the excitotoxic potential of this amino acid [21]. Decreased synthetic rates of glutathione induced by hypoosmolality may therefore foster excitotoxic injury from accumulated glutamate.

Taurine, which is important to the normal maturation of the central nervous system in cats and pre-term human infants [28, 29], may also be an important antioxidant in brain tissue. Taurine is known to ameliorate oxidative injury in lung epithelial cells and erythrocytes [6, 8], and to reduce injury in puromycin aminonucleoside nephrosis, a model of renal disease mediated by reactive oxygen species [9]. Taurine reduces hypoxic injury in rat hippocampal slices [13], but it is unclear if this finding is due to a direct antioxidant effect of the amino acid or to taurine's modulation of excitotoxic injury [10, 30]. The relatively low levels of antioxidant enzymes such as catalase and superoxide dismutase in brain increase the potential importance of the antioxidants glutathione and taurine in this tissue [31, 32]. Moreover, agents which facilitate injury caused by reactive oxygen species, such as iron and

ascorbate, exist at high concentrations in brain [33, 34]. Even under basal conditions, glutathione and taurine may serve an important antioxidant role, since Sinet, Heikkila and Cohen have demonstrated generation of H_2O_2 by normal rat brain *in vivo* [35].

To investigate whether osmotic depletion of glutathione and taurine from brain cells increases their susceptibility to oxidative injury, we exposed cultured brain cells to H_2O_2. Once adapted to hypoosmolar media, SK-N-SH cells were more susceptible to cytolytic injury induced by H_2O_2 than were cells maintained in isosmolar media. Miyamoto and co-workers demonstrated that glutathione protects neuronal cells from oxidative injury imposed by excitotoxic amino acids [36]. Interestingly, hypoosmolar C6 glioma cells were not more susceptible to H_2O_2 than were C6 cells grown in isosmolar media. Enhanced vulnerability of SK-N-SH cells compared to C6 cells was seen in isosmolar conditions and became more significant under hypoosmolar culture conditions. Osmotic depletion of glutathione and taurine may contribute to this enhanced susceptibility of neuronal cells to oxidative injury. The relative resistance of C6 cells to oxidative injury may be due to the higher levels of glutathione in C6 cells in both isosmolar and hypoosmolar media, thereby providing greater protection from H_2O_2-induced injury. Higher levels of glutathione in glial cells than in neuronal cells has previously been reported in primary cultures of these cell types [37, 38]. In our *in vivo* studies, the contribution of glutathione to the overall osmotic adaptation of brain to hyponatremia is quite small (~0.5 mM). The importance of this reduction in brain glutathione content is related to the loss of antioxidant capacity and not from glutathione's minor contribution to volume regulation. Taurine is a quantitatively more important solute in volume regulation with its concentration in brain water falling approximately 3.5 mM in response to hyponatremia.

Chronic hyponatremia constitutes one of the most common disturbances of fluid and electrolyte balance in clinical medicine [39, 40]. In subarachnoid hemorrhage, a condition frequently complicated by hyponatremia, Hasan, Wijdicks and Vermeulen reported a significantly greater incidence of cerebral infarction in those patients who developed hyponatremia when compared to those who remained normonatremic [41]. Ischemic injury in this setting could be related to depletion of brain antioxidants by hyponatremia. Another complication of hyponatremia reported in both animal models and humans is the development of demyelinating brain lesions following the overly rapid correction of chronic hyponatremia [1, 2, 42–44]. In a rat model, Mickel, Oliver and Starke-Reed demonstrated increased oxidation of brain protein in rats subjected to rapid correction of hyponatremia [45]. This finding implicates a role for oxidative injury in the osmotic demyelination syndrome. Osmotic depletion of antioxidants could contribute to this phenomenon.

Considered together, our findings *in vivo* and *in vitro* identify hypoosmolality-induced depletion of brain glutathione and confirm previous reports of the osmolyte function of taurine. While contributing to the volume adaptation of brain during hyponatremia, loss of these antioxidants may be maladaptive when viewed from the perspective of defense against oxidative stress.

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