Taurine and Osmoregulation: Taurine Is a Cerebral Osmoprotective Molecule in Chronic Hypernatremic Dehydration¹

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ABSTRACT. We studied the effect of 8-wk dietary taurine depletion on the vulnerability to hypernatremic dehydration in postweanling kittens. While experimental taurine depletion was not associated with increased susceptibility to acute hypernatremia (1.5 M NaCl/NaHCO₃, 35 ml/kg body weight, single injection), there was an increase in mortality (five of seven *versus* one of seven, $p = 0.05$) and seizure activity (three of seven *versus* none of seven, $p = 0.08$) in taurine-depleted compared to taurine-replete kittens rendered chronically hypernatremic over 96 h. Furthermore, there was a significant decrease in brain cell water (517.4 ± 21.7 *versus* 671.6 ± 32.3 ml/100 g fat-free dry weight, $p < 0.05$), derived almost exclusively from the intracellular compartment (352.5 ± 12.3 *versus* 483.8 ± 34.6 ml/100 g fat-free dry weight, $p < 0.05$) that correlated with the reduction in the cerebral taurine content in the taurine-depleted *versus* control kittens during chronic hypernatremic dehydration. These results suggest that taurine is an important cerebral osmoprotective molecule. This amino acid constitutes nearly 50% of the adaptable intracellular osmolal pool whose concentration varies in the course of osmoregulating in response to perturbations in the extracellular fluid tonicity. (*Pediatr Res* 23: 35–39, 1988)

Abbreviations

CHD, chronic hypernatremic dehydration
CSAD, cysteinesulfinic acid decarboxylase

Recent evidence suggests that in many animal species taurine, a β -aminosulfonic acid formed in the metabolism of methionine and cysteine or present in the diet, acts as an osmoprotective molecule under stressful conditions (1). Hypernatremic dehydration is a formidable problem in clinical pediatrics. Rapid onset of hypernatremia and hasty rehydration are associated with the occurrence of cerebral shrinkage or swelling, respectively, caused by two distinct phenomena (2). The first is the slow diffusion of solute through the "blood brain barrier" so that an osmolal gradient will be initially relieved by the movement of water alone causing a volume change in the brain. The cerebral volume change is gradually attenuated by a second process that is oper-

ative during more prolonged hypernatremia, namely the intracellular accumulation of osmoprotective molecules. These solutes, previously referred to as "idiogenic" (3), consist in part of amino acids, taurine in particular (4). If rapid rehydration is attempted, these molecules cannot exit from the cells quickly enough and cellular swelling may ensue due to the sudden reversal of the osmolal gradient.

The present studies were designed in kittens to assess the role of taurine as a cerebral osmoprotective molecule in CHD. These animals synthesize little taurine owing to low levels of CSAD activity (5, 6). In addition, the cat uses taurine to synthesize the bile salt taurocholate and is unable to switch to glycocholate under conditions of taurine deprivation (7). The cat is, therefore, dependent on dietary sources for taurine (5, 6), and is a useful model for study of the role of taurine as an osmoprotective molecule.

METHODS

Domestic kittens obtained from a breeding colony at the Animal Research Facility of the State University of New York Health Science Center at Brooklyn, and ranging in weight from 600 to 800 g, were fed an experimental casein-based taurine-free diet or a control diet supplemented with 0.05% taurine (BioServ, Inc., Frenchtown, NJ). Control animals differed from "experimental" animals only in the diet ingested for the 8-wk preparatory period. The diets were provided in color-coded pellet form beginning at 8–10 wk of age and continued for 8 wk. Body weight was measured weekly to ensure adequate food intake and growth by the kittens. Water was provided *ad libitum* during the dietary preparation. After 8 wk, groups of kittens were subjected to the following protocol to induce CHD. On the day of the study, after the animals were weighed, a venous blood sample was drawn for determination of serum osmolality and chemical analyses including plasma taurine concentration. Fluid intake was then totally restricted for 24 h, after which a repeat blood specimen was removed for measurement of serum osmolality and chemical analyses. For the next 72 h, the only fluid provided was thrice daily 1 M NaCl solution via orogastric feeding, a dose designed to raise the serum Na⁺ concentration gradually to 180, 185, and 190 mmol/liter on days 2, 3, and 4, respectively (8). All animals were observed several times daily during the dehydration phase for seizure activity or other abnormal neurological behavior. The kittens were immediately dissected at the time of death or sacrificed and dissected 96 h after commencing the dehydration protocol. In no instance did an animal remain in its cage for longer than 15 min after dying and before dissection. Blood samples were retained for measurement of serum osmolality and chemical analyses. A segment of the brain from the frontal lobe and a portion of the vastus medialis muscle were rapidly excised

Received May 15, 1987; accepted August 28, 1987.

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¹ Presented at the annual meeting of the Society for Pediatric Research on May 7, 1986 in Washington, D.C.

within 15 min of sacrifice or death. Each tissue specimen was divided into three segments for determination of percentage water content, tissue electrolyte concentrations, and taurine content, respectively.

Analytic methods. Serum osmolality was determined using a freezing point depression osmometer (Advanced Instruments, Needham, MA) and chemical analyses were performed using a Klineflame automated analyzer (Beckman Instruments, Brea, CA). The brain and muscle tissue specimens were dried to constant weight in an oven at 40° C for determination of percentage water content. The specimens used for tissue electrolyte concentrations were first extracted twice in ether and redried to constant weight. The tissues were digested in 8 volumes of a 5 N HNO₃ solution for 72 h and the extracts were then neutralized with CaCO₃. Measurements of sodium and potassium contents were conducted using an automated analyzer, while tissue chloride content was determined using a chloridometer (Buchler, Saddle River, NJ). The brain and muscle tissue samples used for determination of taurine concentration were homogenized in 9 volumes of cold 10% trichloroacetic acid using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) and centrifuged at 20,000 × *g* for 30 min. The plasma and tissue taurine contents were determined using an automatic amino acid analyzer (Beckman Instruments) on-line to a computer (Spectra Physics, Oxnard, CA) for data acquisition and calculation.

Calculations. The extracellular water content of the tissue samples was calculated as the chloride space. The latter was determined by dividing the tissue chloride content by the chloride concentration corrected by the Donnan factor (0.96) and for the water content of plasma (0.93) (3). The intracellular water content was derived as equal to the total tissue water content minus the extracellular water component (3).

Statistical methods. The results were analyzed using Fisher's exact test and Student's *t* test, paired or unpaired, as dictated by the experimental conditions. Results were considered statistically significant if *p* < 0.05.

RESULTS

All kittens adjusted to the pelleted diets and ingested an adequate daily amount of food to sustain normal growth. As demonstrated in Table 1, a severe state of hypernatremic dehydration was successfully induced during the protocol. The discrepancy consistently observed (10 to 59 mosmol/kg) between the serum Na⁺ concentration and osmolality may be a reflection of the hyperglycemia associated with hypernatremic dehydration (2, 9). The lower serum Na⁺ and osmolality achieved in the control animals during CHD probably resulted from improved survival and renal excretion of the saline load in the 16-h interval between the final dose of 1 M NaCl and the time of sacrifice. However, this could not be documented since urinary sodium excretion was not measured during the induction of hypernatremic dehydration.

The body weights of the kittens at the start of the dehydration phase were comparable on the two diets in the experimental protocol (Table 2). However, more severe dehydration was seen in the control *versus* experimental animals reflecting the improved survival of the taurine-replete kittens during the dehydration regimen. Despite the increased severity of total body dehydration in the control kittens, the animals raised on the experimental taurine-free diet manifested increased early mortality (five of seven experimental *versus* one of seven, control, *p* = 0.05) and a higher incidence of generalized tonic-clonic seizures (three of seven experimental *versus* none of seven, control, *p* = 0.09).

In parallel with this enhanced susceptibility to the clinical consequences of CHD in the taurine-deficient kittens, there was a significant reduction in percentage brain water content in experimental *versus* control animals, 73.4 ± 0.5 *versus* 76.7 ± 0.3, respectively (*p* < 0.005) (Table 3). No differences were noted

in the percentage water content of muscle specimens obtained from these animals.

Table 3 summarizes the changes in extracellular and intracellular water content of the brain and muscle specimens obtained from the taurine-depleted and control animals with CHD. In brain, a significant reduction in water content, derived almost exclusively from the intracellular compartment, was detected in the taurine-deficient kittens compared to control taurine-replete animals. A similar pattern was not demonstrable in muscle tissue.

The intracellular concentrations of Na⁺ and K⁺ are shown for the same animals in Table 4. While no significant differences in intracellular Na⁺ and K⁺ (mmol/liter) concentrations of the brain and muscle were seen, intracellular Na⁺ concentrations tended to be higher in experimental *versus* control animals. In most

Table 1. Initial and final serum sodium concentration and osmolality achieved during CHD (mean ± SEM)*

	Experimental (n = 7)	Control (n = 7)
Sodium (mmol/liter)		
Initial	149 ± 1.2	146 ± 1.8
24-H	153 ± 0.4	152 ± 0.7
Final	183 ± 4.0†	177 ± 2.1†
Osmolality (mosmol/kg)		
Initial	311 ± 2.1	309 ± 1.1
24-H	317 ± 1.3	314 ± 1.5
Final	425 ± 12.3†‡	376 ± 9.1†

* Abbreviations: E, experimental (taurine depleted); C, control (taurine replete).

† *p* < 0.001 *versus* initial value.

‡ *p* < 0.01 *versus* control value, final.

Table 2. Percentage dehydration, mortality, and morbidity in CHD (mean ± SEM)*

	Experimental (n = 7)	Control (n = 7)
Body wt (g)		
Initial	1896 ± 123	1934 ± 62
Final	1661 ± 118	1553 ± 56
% Weight Loss	13.3 ± 3.0	19.8 ± 1.2
Mortality	5/7	1/7†
Seizures	3/7	0/7

* Abbreviations: E, experimental (taurine depleted); C, control (taurine replete).

† *p* = 0.05, E *versus* C.

Table 3. Brain and muscle water content and fluid compartment sizes in animals with CHD (mean ± SEM)*

	% H ₂ O Content	TTW†	ICW†	ECW†
Brain				
E (n = 7)	73.4 ± 0.5	517 ± 21.7	353 ± 12.3	165 ± 22.5
C (n = 7)	76.7 ± 0.3‡	672 ± 32.3§	484 ± 34.6§	187 ± 13.0
Muscle				
E (n = 7)	61.0 ± 1.1	239 ± 17.1	133 ± 12.2	106 ± 15.3
C (n = 7)	63.8 ± 1.7	284 ± 34.0	139 ± 26.3	145 ± 15.7

* Abbreviations: E, experimental (taurine depleted); C, control (taurine replete); TTW, total tissue water; ICW, intracellular water; ECW, extracellular water.

† All tissue water compartments are expressed as ml/100 g fat-free dry weight.

‡ *p* < 0.005 E *versus* C.

§ *p* < 0.05 E *versus* C.

Table 4. Tissue intracellular fluid electrolyte concentrations in animals with CHD (mean $\pm$ SEM)*

	Brain		Muscle	
	Na ⁺ (mmol/liter)	K ⁺ (mmol/liter)	Na ⁺ (mmol/liter)	K ⁺ (mmol/liter)
E	7.4 $\pm$ 3.5 (n = 7)	110 $\pm$ 10.4 (n = 5)	23.0 $\pm$ 10.1 (n = 7)	153 $\pm$ 13.1 (n = 5)
C	1.0 $\pm$ 1.0 (n = 7)	124 $\pm$ 9.2 (n = 6)	8.9 $\pm$ 5.0 (n = 6)	130 $\pm$ 8.2 (n = 4)

* Abbreviations: E, experimental (taurine depleted); C, control (taurine replete).

previous experiments (3), the sodium space was either smaller than or equal to the chloride space, so that the derived Na⁺ concentration in the intracellular fluid is close to 0. The taurine-depleted kittens in protocol II had a mean brain intracellular fluid Na⁺ concentration of 7.4 mmol/liter.

The tissue taurine content of brain and muscle specimens removed from animals with chronic hypernatremic dehydration are summarized in Table 5. The mean taurine content was lower in both tissues in the experimental animals maintained on the taurine-free diet, attesting to the adequacy of the 8-wk dietary preparatory phase in inducing a state of taurine depletion. However, the reduction was statistically significant only in the muscle samples. The difference in brain taurine content was more pronounced if the values obtained in the 10 animals that survived at least 72 h of the dehydration protocol are compared to the results seen in the animals that died prior to this time (12.1 $\pm$ 3.2 *versus* 1.9 $\pm$ 0.2 mmol/kg fat-free dry weight, $p < 0.01$). In addition, a significant correlation was noted between brain taurine content and percent cerebral water content when the data from all kittens were analyzed together ($Y = 72.9 + 0.27 X$, $p < 0.02$) (Fig. 1).

DISCUSSION

These studies demonstrate that taurine functions as a cerebral osmoprotective molecule, lessening brain cell desiccation during chronic hyperosmolar dehydration states. It is postulated that sustained extracellular hypertonicity acts as a stimulus to increase the intracellular taurine concentration by triggering its release from an internal solute reservoir. Acting as one of the previously hypothetical "idiogenic osmoles," the increased cerebral taurine concentration decreases the extracellular to intracellular osmolal gradient and attenuates brain cell shrinkage. Although this proposal is a negative inference based on a heightened susceptibility to sustained hypertonicity in taurine-depleted animals, it is supported by the parallel reduction in brain water and taurine content and the consistent lack of a similar effect in muscle, a representative extracerebral tissue.

While untreated, normonatremic taurine-replete controls were not investigated, the clear differences in survival and brain cell water compartment sizes between simultaneously studied taurine-deficient and taurine-replete kittens are adequate to support our conclusions. It is the magnitude of the differences and not the absolute values of the cerebral water compartment sizes that argue in favor of a vital osmoprotective role for taurine. The lack of apparent change in muscle requires further investigation; it is known from previous work that osmoprotective molecules also appear in muscle tissue (3). Our failure to observe changes may reflect only smaller differences which, owing to the small number of animals studied here, makes the muscle tissue a less sensitive indicator of the phenomenon.

At first glance the data accumulated during CHD of the experimental animals suggest an exaggerated contraction of their cerebral intracellular water compartment because of a more severe hyperosmolar state. However, closer examination reveals

Table 5. Tissue taurine contents in animals with CHD (mean $\pm$ SEM)*

	Brain†	Muscle†	Plasma‡
E	5.6 $\pm$ 1.9 (n = 7)	4.9 $\pm$ 0.8§ (n = 7)	11.2 $\pm$ 2.1 (n = 7)
C	9.5 $\pm$ 1.9 (n = 7)	9.6 $\pm$ 1.3 (n = 7)	11.0 $\pm$ 1.8 (n = 7)

* Abbreviations: E, experimental (taurine depleted); C, control (taurine replete).

† Results reported as mmol/kg fat-free dry weight.

‡ Results reported as μ mol/100 ml.

§ $p < 0.05$, E *versus* C.

Fig. 1. The linear relationship between brain taurine content and percentage brain water content in the kittens subjected to chronic dehydration (●, experimental; ○, control) 15 shown.

that the serum Na⁺ concentrations were comparable in the experimental and control groups. Furthermore, despite a lower serum osmolality in the control animals, they survived for a significantly longer period of time which should maximize the contribution of the intracellular water compartment to total body fluid deficits. Finally, while there was a statistically significant correlation between brain cell water and taurine content, no such relationship existed between brain cell water and serum osmolality. A great deal of the variability in the degree of hyperosmolality and hypernatremia in the control animals with CHD resulted from the relatively premature death of these animals.

A number of studies have indicated a substantial reduction (80–90%) in cerebral taurine content of kittens after 8–10 wk on a taurine-free diet and our results are compatible with these reports (6, 10–12). Depletion of brain taurine stores may be subject to interanimal variation. This may account for the higher brain taurine content and survival of the two experimental kittens during the chronic dehydration protocol, despite 8 wk of total absence of taurine from their diet. This observation actually strengthens the assertion that taurine acts specifically within the brain as an osmoprotective solute since muscle taurine deficiency was observed in all experimental animals and no difference in the severity of muscle dehydration was observed in experimental *versus* control animals.

A note of explanation regarding the use of chloride space as a marker of extracellular water is in order at this point. Although newer methods for estimating extracellular water volume have been devised [radiolabeled inulin or polyethylene glycol (13)], there are difficulties with these techniques. Both indicators require a prolonged, continuous infusion to enable adequate distribution of the marker in the extracellular fluid space (14), which would have been a difficult procedure in extremely ill,

dehydrated animals. Because chloride may not be entirely excluded from the cerebral cell (15), the true anatomic meaning of the chloride space in the brain has been a subject of much dispute. However, we estimated the size of the "intracellular" water compartment in an equivalent manner in both groups of animals in protocol II. Early comparisons of chloride and inulin spaces actually favored chloride as a superior marker of the actual extracellular space (16). In addition, if taurine uptake by the brain, a sodium and chloride dependent cotransport process (17, 18), contributes to intracellular chloride content, then this flux should be enhanced in the control animals. This would result in underestimation of the brain intracellular water in the taurine-replete animals with CHD and strengthens rather than detracts from our conclusion.

A role for taurine in osmoregulation is supported by previous findings in amphibians and other subvertebrate species in which alterations in brain taurine concentration have been seen during different phases of the animal's life cycle and associated with changes in the tonicity of the ambient environment (19, 20). In addition, a nearly 2-fold rise in brain taurine concentration has been observed in rats and mice rendered hypernatremic (4, 21, 22). The lack of any deleterious effect of dietary taurine deficiency in acute hypernatremia (single injection of 1.5 M NaCl/NaHCO₃ 35 ml/kg body weight, Trachtman H, *et al.* unpublished observations), is consistent with experimental work demonstrating that at least 4 h of sustained hypernatremia are required to stimulate the formation of osmoprotective "idiogenic solutes" (23).

Based on our findings, a quantitative assessment of the contribution of taurine to brain intracellular osmolality suggests that the taurine concentration in the intracellular water compartment is only 3–5 mmol/liter. However, if taurine is confined to astrocytes which are assumed to comprise 30% of brain volume, then its intracellular concentration can be estimated to be nearly 10–15 mmol/liter and may constitute up to 50% of the newly generated free intracellular amino acid solutes in response to prolonged extracellular hypertonicity. These calculations are consistent with the observations cited earlier that the rise in cerebral intracellular taurine concentration accounts for half of the increment in brain amino acid content during experimental hypertonic states (4, 21, 22). Thus, although inorganic ions such as K⁺ make a larger quantitative contribution to cell volume regulation than organic solutes, and taurine constitutes less than 10% of the total intracellular osmolal content during sustained hypernatremia, the adaptive increase in its concentration may be critical in regulating cerebral cell volume.

Van Gelder and coworkers (24, 25) have postulated that intragial cell taurine acts to control central nervous system water balance by additional means, *i.e.* modulating astrocyte carbonic anhydrase and glutamate dehydrogenase activities. The importance of astrocytes in regulating brain water content is suggested by the close proximity of glial cells and cerebral blood vessels and the finding of astrocyte swelling in many disease states accompanied by cerebral edema (26). Thus, while quantitatively the astrocyte does not constitute the bulk of cerebral cell mass or tissue water, it appears to be strategically located to control neuronal cell water content. Finally, it is possible that the discrepancy between the profound cerebral osmoprotective effect of taurine and its less impressive increase in intracellular content is explained by an additional mechanism of action in conjunction with an expansion of the cytosolic pool of osmoprotective solutes. Just as in the lung where taurine protects the pulmonary epithelium against nitrogen dioxide injury by preserving tight junction integrity (27), taurine may exert its cerebral osmoprotective function by maintaining the endothelial membrane structure of the blood brain barrier and limiting water movement during hypertonic stress. This proposal is consistent with experiments in which hypernatremia has been shown to disrupt the blood brain barrier (28).

It is possible that other constituents of the pool of "idiogenic osmoles" such as glutamine, glutamate, aspartate, glycine, and

γ -aminobutyric acid (29, 30) may play a larger osmoprotective role during dietary taurine depletion. However, measurements of brain amino acid content using specific microenzymatic assays in the tissue homogenates obtained from the experimental and control animals with CHD revealed no differences in cerebral aspartate and glutamate plus glutamine contents. The increase in brain and muscle intracellular fluid Na⁺, while not statistically significant, may represent a compensatory response in cellular inorganic ion concentration to sustained hypernatremia in kittens with taurine deficiency. In a number of cell types, the regulatory volume increase provoked by incubation in a hypertonic medium is mediated by Na⁺-H⁺ antiport exchange (31). An additional mechanism to increase cerebral intracellular concentration of taurine and Na⁺ in control taurine-replete animals is the linked cotransport of these two solutes by brain synaptic membranes (32, 33).

The increased frequency of seizure activity in the taurine-depleted kittens during chronic hypernatremic dehydration is consistent with evidence that taurine acts within the central nervous system as an inhibitory neurotransmitter (24, 34). This membrane-stabilizing function of taurine may only be manifested during stressful circumstances that lower the seizure threshold such as the CHD regimen.

Further work will be required to determine whether taurine acts similarly as a cerebral osmoprotective molecule in hypertonic states due to sustained hyperglycemia or chronic uremia. Of note is the observation that the brain taurine content is nearly doubled in the brain tissue removed from rats with chronic renal failure following 5/6 nephrectomy (35). In addition, rapid intravenous rehydration studies are needed to ascertain whether taurine deficiency is associated with lesser neurological morbidity during fluid correction due to a lower content of osmoprotective osmoles leading to rebound cerebral edema. Whether supplementation with lipid soluble taurine derivatives in early phases of hypernatremic dehydration would decrease the severity of neurological sequelae merits further attention (36). Finally, the applicability of these studies to human infants remains to be investigated.

Acknowledgments. The authors express their gratitude to Dr. Jean Holowach-Thurston who kindly performed the measurements of cerebral glutamate and aspartate in the kittens with CHD. In addition, we thank Mrs. Barbara Ho for her excellent help in preparing the manuscript, and Kishore Phadke, M.D. and Ms. Mabel Wong for their technical assistance in performing the experiments.

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