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myo-Inositol: A Newly Identified Nonnitrogenous Osmoregulatory Molecule in Mammalian Brain

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ABSTRACT. Sugar alcohols have been found to play an important osmoregulatory role both in unicellular organisms and, more recently, in multicellular organisms, including mammals. This study shows that myo-inositol accumulates in the brains of chronically hypernatremic mice, as had been earlier found in rats, and demonstrates for the first time a profound decrease of myo-inositol in the brains of chronically hyponatremic mice. Together with decreases in better known cerebral osmoles (amino acids and related nitrogenous compounds), the decrease in myo-inositol apparently allows the brain to balance its intracellular osmolality with that of the plasma, permitting a normal brain water content (no edema) despite profound hyponatremia. (*Pediatr Res* 26:482–485, 1989)

Cellular adaptation to osmotic stress is a vital biologic process that protects organisms from the possibly lethal effects of dehydration and shrinkage, or edema and swelling of cells. In 1955 McDowell *et al.* (1) described the generation of unidentified ("idiogenic") osmotically active particles other than electrolytes in mammalian tissues in response to treatment with hyperosmolar solutions. Since then a large number of studies have appeared concerning the cellular adaptation of different species to osmotic stress. Duchateau *et al.* (2) were the first to show that loss or gain of free tissue amino acids played a prominent role in the adaptation of invertebrates to extremes of environmental salinities. The role of amino acids and related compounds in adaptation to salinity change has since been extended to bacteria (3), plants (4, 5), amphibians (6), and mammals (7–11). Not all amino acids contribute equally to cellular osmoregulation. For example, on a molar basis the greatest concentration change during salinity stress was in alanine in bivalve molluscs (12), glycine in lobsters (13), glutamate in toad (14), and taurine in mouse brain (9, 10). Also, not all osmoregulators are of nitrogenous origin. In osmophilic yeasts the predominant osmoregulatory molecule is arabitol (15), in green algae, it is glycerol (16). Recently a significant role of sugar alcohols in osmoregulation in higher species has been recognized. Pinitol and myo-inositol have been found to accumulate in drought-adapted varieties of maritime pine during water stress (17). Bagnasco *et al.* (18) found increased levels of myo-inositol and sorbitol in rat and rabbit renal medullas during antidiuresis. Of especial interest to us, because of the important role of myo-inositol and the phosphoinositides in neural transmission, was the finding of Lohr *et al.* (19) that myo-inositol levels are increased in the brains of chronically hypernatremic rats. This was the first report citing an increase in inositol levels in brain and raises the important question of whether that change is reflected in the inositol-containing lipids and in their function.

We have earlier reported on the effects of chronic hyper- and hyponatremia and the effect that rapid restoration of plasma Na^+ levels to normal has on brain water, electrolyte, carbohydrate, energy and amino acid metabolism in young mice (10, 11). It was thus of interest to confirm the findings of Lohr *et al.* (19) in the chronic hypernatremic state and to examine the effect of chronic hyponatremia, and rapid correction, on brain myo-inositol levels.

MATERIALS AND METHODS

Preparation of animals. Litters of normal 19- to 29-d-old Swiss Webster mice were used because of our clinical and research interest. In each experiment, control and experimental animals were weight-matched littermates.

Chronic hypernatremia and rehydration. Hypernatremia was produced by water deprivation and intraperitoneal injections of 1 M NaCl, 20 mL/kg, once or twice daily (seven doses total) for 4 d; laboratory food was allowed *ad libitum.* Control littermates were allowed water and food and received an equivalent volume and number of injections of sterile 0.9% NaCl. On the fourth day, 2 h after the last injection, controls and one-half of the experimental animals were killed by decapitation. The remaining experimental mice were rehydrated over the next 4 h by three subcutaneous injections of 2.5% dextrose in water, 100 mL/kg each dose, given at 80-min intervals. Animals were decapitated 80 min after the last injection of the glucose solution.

Chronic hyponatremia and correction with saline. Chronic hyponatremia was induced in fasted and thirsted mice by intraperitoneal injection of 50 mL/kg of 2.5% dextrose in H_2O and subcutaneous injection of 5 U/kg of vasopressin tannate (Pitressin) in peanut oil once daily for 3 d. There were two groups of normonatremic controls. One group was allowed food and water *ad libitum.* The second group was fasted and thirsted for 3 d. Both groups received daily injections of 0.9% NaCl, intraperitoneally and subcutaneously, equal in volume to the 2.5% dextrose in H_2O or vasopressin tannate given the hyponatremic animals (see above). At 0800 h on the fourth day, blood was collected from the snipped tail of some of the experimental mice for plasma Na^+ levels, after which the animals received one intraperitoneal injection of 1 M NaCl, 20 mL/kg, followed by two subcutaneous injections of 0.9% NaCl (one at 1100 h, the other at 1500 h), 30 mL/kg each time. Animals were killed at 1700 h, 9 h after the onset of treatment. Both groups of controls and the remaining untreated hyponatremic mice were decapitated in the afternoon of the fourth day. At decapitation the head was allowed to drop directly into liquid N_2 with rapid stirring. Blood was

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collected from the severed neck vessels in heparinized microcapillary tubes (Caraway, American Hospital Supply Corp., McGaw Park, IL). Frozen heads were stored at −80°C until the time of dissection and preparation of tissue extracts.

Preparation of plasma and brain. Blood was centrifuged promptly at 4°C. Fresh plasma was used for electrolyte determinations. Frozen brain anterior to the posterior colliculi was dissected free of meninges and visible blood vessels in a cryostat at −35°C. The brains were powdered and weighed in a cold room at −22°C and perchloric acid extracts were prepared at 4°C (20). The extracts were stored at −80°C until the time of the assay.

Analytical methods. The Na^+ concentration in fresh plasma was determined in a flame photometer. myo-Inositol levels were measured as follows: 25-μL aliquots of brain perchlorate extracts were lyophilized and treated with 100 μL of dry pyridine:bis(trimethylsilyl)trifluoroacetamide (Regis Chemical Co., Morton Grove, IL) 1:1 (v:v) containing 50 pmol of [$^2H_6$]myo-inositol (MSD Isotopes, Montreal, Canada) as internal standard. After 24 h 1-μL aliquots were analyzed on a Hewlett-Packard model 5970 gas chromatograph/mass spectrometer (Hewlett-Packard Co., Palo Alto, CA) using fragment ions m/z 318 for the endogenous myo-inositol and m/z 321 for the internal standard. Chromatography was on a 15 meter DB-17 column (J & W Scientific, Folsom, CA) at 190°C using a split ratio of 10:1.

Statistical analysis. The statistical significance of the difference between the mean values of the findings in two groups of mice was determined by Student's *t* test. When findings from three groups of mice were compared, statistical significance was first determined by a one-way analysis of variance followed by the HSD method of Tukey (21) to identify statistically significant differences between specific pairs of means.

RESULTS

The myo-inositol level in the brains of three different litters of normal 19- to 29-d-old mice (Tables 1–3) was similar to that reported by Stewart *et al.* (22) for normal adult rat brain. In adult rats Lohr *et al.* (19) found brain myo-inositol levels that were twice as high; we have no explanation for this discrepancy, however other laboratories have reported rat brain inositol levels similar to ours. For example Palmano *et al.* (23), using gas chromatography, as did Lohr *et al.* (19), reported control rat brain myo-inositol levels to be 6.62 ± 0.96 mmol/kg wet wt (n = 14) and MacGregor and Matschinsky (24), using an enzymatic fluorometric assay, reported rat brain myo-inositol levels to be 5.8 ± 0.2 mmol/kg wet wt (n = 6).

Chronic water deprivation and salt loading of young mice induced a 43% increase in the brain myo-inositol concentration (Table 1). This increase was roughly equal to that found in chronically hypernatremic adult rats (19) and was quantitatively similar to the adaptive increases in levels of amino acids in these same animals (10). Rapid rehydration of the hypernatremic mice returned the plasma Na^+ concentration to normal but had no effect on the elevated brain myo-inositol content. The total sum of the measured cerebral amino acids was also still significantly higher after treatment than in the brains of normonatremic controls (10).

Inasmuch as the animal model of chronic hyponatremia necessitated withdrawal of food and water throughout the experimental interval it was important to examine the effect of these restrictions in normonatremic littermates (Table 2). The stringent experimental conditions induced a 4% increase in the plasma Na^+ concentration (probably a reflection of dehydration). Despite a wt loss of 26% (equal to that of the hyponatremic mice) there was no change in the brain myo-inositol concentration. In the brains of chronically hyponatremic mice the myo-inositol concentration fell 88% (Table 3). By comparison levels of amino acids in these brains decreased 35 to 67% (11). Rapid elevation of the depressed plasma Na^+ level to normal with saline did not increase the brain myo-inositol content significantly and the sum of the measured amino acids and creatine in these same animals (11) was still reduced one-third or more. As a result rapid correction of chronic hyponatremia caused cellular dehydration and shrinkage of the brain (11).

Table 1. *Effect of chronic hypernatremia and rapid correction on plasma Na^+ and brain myo-inositol levels in young mice (mean ± SE)**

Measurement	Control (n = 6)	Chronic hypernatremia: Untreated (n = 9)	Chronic hypernatremia: Rapid correction (n = 4)
Plasma Na^+ (mEq/L)	148	191	151
Brain myo-inositol (mmol/kg)	4.84 ± 0.46	6.91 ± 0.24†	6.07 ± 0.32†

* Treatment of animals (number in parentheses) and other procedures are given in "Materials and Methods." Plasma Na^+ levels are taken from Reference 10.

† $p < 0.01$ *versus* control.

Table 2. *Effect of chronic fasting and thirsting on plasma Na^+ and brain myo-inositol levels in young normonatremic mice (mean ± SE)**

Measurement	Fed controls (n = 4)	Fasted and thirsted controls (n = 6)
Plasma Na^+ (mEq/L)	142	148
Brain myo-inositol (mmol/kg)	5.31 ± 0.30	4.46 ± 0.54

* Preparation of animals (number in parentheses) and other procedures are given in "Materials and Methods." Plasma Na^+ levels are from Reference 11.

Table 3. *Effect of chronic hyponatremia and rapid correction on plasma Na^+ and brain myo-inositol levels in young mice (mean ± SE)**

Measurement	Fasted and thirsted controls (n = 6)	Chronic hyponatremia: Untreated (n = 10)	Chronic hyponatremia: Rapid correction (n = 8)
Plasma Na^+ (mEq/L)	148	91	134
Brain myo-inositol (mmol/kg)	4.46 ± 0.54	0.54 ± 0.12†	0.87 ± 0.10†

* Treatment of animals (number in parentheses) and other procedures are given in "Materials and Methods." Plasma Na^+ levels are from Reference 11.

† $p < 0.01$ *versus* control.

Changes in the cerebral levels of myo-inositol (and amino acids) seen in chronic hyper- and hyponatremia are not merely a reflection of dehydration of the brain in one case, and edema in the other. Whereas the dry wt of the brain increased some 8 to 11% in chronically hypernatremic mice (7, 10), myo-inositol increased 43% and levels of most amino acids increased 20 to 90% (7, 9, 10). Furthermore, the 88% decrease of the myo-inositol concentration in chronic hyponatremia was seen without any change in the brain water content (11).

DISCUSSION

During adaptation to chronically increased or decreased plasma Na^+ levels, levels of amino acids in the brains of young mice increased or decreased, respectively, to maintain osmotic equilibrium and to limit the loss or gain of water in brain (10, 11). The present data reveal similar paradoxic changes in the content of myo-inositol in the brains of these same animals. The

reciprocal changes in the cerebral levels of myo-inositol in chronic hyper- and hyponatremia and their magnitude establish a role of myo-inositol in the maintenance of intracellular osmoregulation in mammalian brain comparable to that of amino acids and related nitrogenous compounds. For example, in the brains of chronically hypernatremic mice individual levels of six amino acids with a concentration of more than 1 mmol/kg (aspartate, glutamate, glutamine, GABA, glycine, taurine) increased 0.52 to 8.25 mmol/kg, mean 2.32 mmol/kg (9). By comparison the myo-inositol content increased 2.07 mmol/kg. In the chronically hyponatremic mice cerebral levels of these same amino acids decreased 0.78 to 5.82 mmol/kg, mean 3.36 mmol/kg (11), whereas the myo-inositol level fell 3.92 mmol/kg.

The mechanism of the adaptive changes in levels of amino acids and myo-inositol to maintain osmotic equilibrium across the cell membrane is unknown. Na^+ plays a critical role in the uptake of amino acids by brain and spinal cord (25–27) and in the uptake of myo-inositol in sciatic nerve (28) and in a neuroblastoma cell line (29). Thus, the reciprocal changes in the brain amino acid and myo-inositol contents in chronic hyper- and hyponatremia may be related to the respective increases or decreases of the plasma Na^+ concentration. Recently Trachtman *et al.* (30) found that taurine uptake was enhanced in synaptosomes isolated from the brains of hypernatremic rats when compared with control animals. The finding suggests that the cerebral transport of taurine (and possibly other osmolytes) in chronic hypernatremic dehydration reflects intrinsic alterations in cell metabolism or transmembrane uptake. Other explanations are possible.

Although generally not as great as with saline environments, adaptive increases of intracellular amino acids have been observed with sucrose in bacteria (3), toads (31), and mammals (1). Chronic water deprivation per se induced a 3-fold increase in the rate of uptake of amino acids in the rat pituitary neural lobe; plasma Na^+ levels were not given (32).

If similar osmoregulatory changes occur in man (the evolutionary data would suggest that they do), findings in the brains of chronically hyper- and hyponatremic animals may have clinical significance. The danger of precipitating seizures or coma following rapid correction of chronic hypernatremia in patients is well known. If the plasma Na^+ concentration was lowered to normal before the increased cerebral levels of amino acids, myo-inositol (and other osmoles) were reduced to normal, then a reverse osmotic gradient (brain osmolality higher than blood) might be created causing an uptake of water from the extracellular space resulting in cerebral edema.

Overly rapid correction of chronic hyponatremia in man can induce cellular necrosis and demyelination in select symmetric regions of brain—the clinical pathologic syndrome of central pontine myelinolysis (33). In chronically hyponatremic mice adaptive reductions in the brain amino acid (11) and myo-inositol contents (this study) permitted a normal brain water content despite extreme hyponatremia. Rapid treatment with saline restored the plasma Na^+ level to normal but had no significant effect on the depressed myo-inositol level. The brain amino acid content was still one-third less than normal (11). In consequence, rapid correction induced cerebral dehydration and shrinkage of the brain volume. How brain dehydration might cause or contribute to the development of central pontine myelinolysis after rapid correction of chronic hyponatremia in animals (34–37) and in man (33) is unknown.

Together with the previously published observations (18, 19) findings in this study establish a significant role of myo-inositol in the maintenance of intracellular osmoregulation in mammals. The changes in the brain levels of myo-inositol in these studies are remarkable. No condition other than chronic hypernatremia has been described that causes an increase in brain myo-inositol. The largest decrease in the level of brain myo-inositol previously reported was 70%, after treatment of rats with lithium chloride and the centrally active cholinomimetic pilocarpine (38). This is to be compared with the 88% decrease we see in the brains of chronically hyponatremic mice. The effect of these procedures on brain phosphoinositide levels and on the inositol phosphate signalling system is presently under study.

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