CONTROL OF BRAIN VOLUME DURING HYPOOSMOLALITY AND HYPEROSMOLALITY

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1. INTRODUCTION

Hypoosmolality and hyperosmolality are relatively common clinical problems (1). Many different factors contribute to the morbidity and mortality known to occur during states of altered osmotic homeostasis. The most serious complications are associated with pathological changes in brain volume: brain edema during hypoosmolar states and brain dehydration during hyperosmolar states. This chapter will summarize what is known about the changes that occur in brain fluid and solute composition during hypoosmolar and hyperosmolar states, which are responsible for the compensatory process of brain volume regulation. Most experimental and clinical studies have used serum sodium concentration as an indicator of osmolality, and throughout this chapter the terms hyponatremia and hypoosmolality are used interchangeably, as are hypernatremia and hyperosmolality.

2. HYPOOSMOLALITY

Hypoosmolality is well known to cause a variety of neurological symptoms, including disorientation, confusion, obtundation, seizures and death from tentorial herniation, called hyponatremic encephalopathy (4,5), but the incidence and severity of such symptoms in hyponatremic patients is quite variable (6). It is not unusual to find patients with low serum sodium concentrations ($[Na^+]$) who are relatively asymptomatic, while others exhibit severe neurological dysfunction at equivalently low levels of serum $[Na^+]$. Such clinical observations indicate that the brain can successfully adapt to even severe degrees of hypoosmolality in many cases. Knowledge of how the brain regulates

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its volume in response to hypoosmolality has been crucial to understanding this sometimes perplexing spectrum of clinical presentations of hypoosmolar patients.

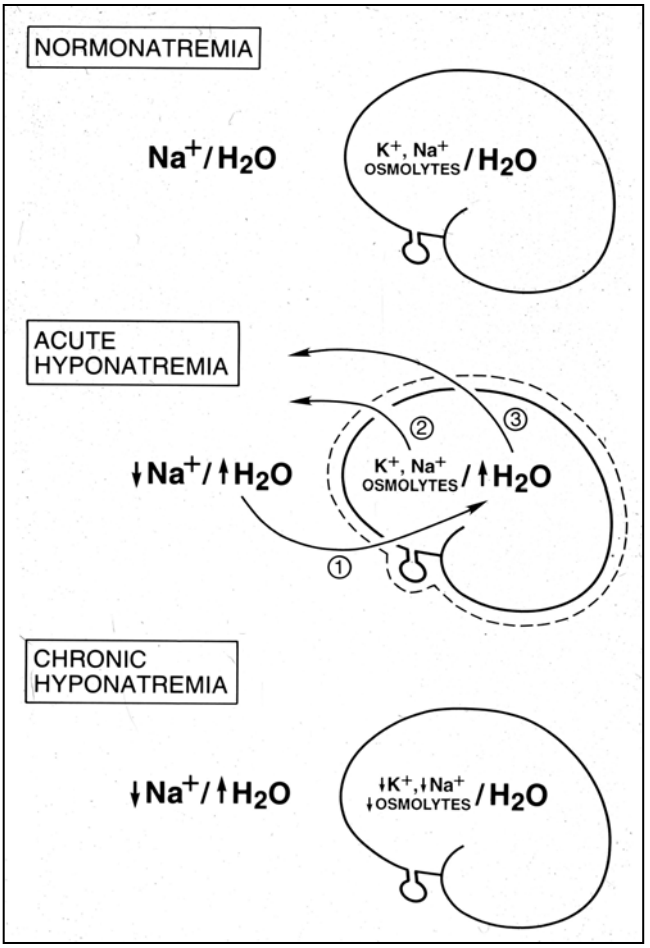

Figure 1. Brain Adaptation to Hypoosmolality: Schematic diagram of brain volume adaptation to hyponatremia. Under normal conditions brain osmolality and extracellular fluid (ECF) osmolality are in equilibrium (top panel; for simplicity the predominant intracellular solutes are depicted as K^+ and organic osmolytes, and the extracellular solute as Na^+). Following the induction of ECF hypoosmolality, water moves into the brain in response to osmotic gradients producing brain edema (middle panel, #1, dotted lines). However, in response to the induced swelling the brain rapidly loses both extracellular and intracellular solutes (middle panel, #2). As water losses accompany the losses of brain solute, the expanded brain volume then decreases back toward normal (middle panel, #3). If hypoosmolality is sustained, brain volume eventually normalizes completely and the brain becomes fully adapted to the ECF hyponatremia (bottom panel). Reproduced with permission from (1).

Experimental studies in animals over the last century have elucidated many of the physiological mechanisms underlying brain adaptation to hypoosmolality (7-9).

Following decreases in plasma osmolality, water moves into the brain along osmotic gradients, causing cerebral edema. In response, the brain loses solute from the extracellular (10) and the intracellular (7,8) fluid spaces, thereby decreasing brain water content back toward normal levels (Fig. 1). The marked variability in the presenting neurological symptoms of hyponatremic patients can be understood in the context provided by this process of brain volume regulation. Most of the neurological symptoms associated with hyponatremia are thought to reflect brain edema as a consequence of osmotic water movement into the brain (5). However, once the brain has volume-adapted through solute losses, thereby reducing brain edema, neurological symptoms will not be as prominent, and in some cases may even be totally absent (Fig. 1).

It has also long been appreciated that the rate of fall of serum [Na^+] is generally more strongly correlated with morbidity and mortality than the actual magnitude of the decrease. This is due to the fact that brain volume regulation occurs over a finite period; the more rapid the fall in serum [Na^+], the more water will be accumulated before the brain is able to lose solute, and with it the increased water. This temporal association explains the much higher incidence of neurological symptoms in patients with acute hyponatremia compared to those with chronic hyponatremia. It is therefore important to understand the mechanisms underlying brain volume regulation during both acute and chronic hypoosmolality.

2.1. Adaptation to Acute Hypoosmolality.

The clinical distinction between acute and chronic hypoosmolality is somewhat arbitrary, but generally hypoosmolality is considered to be acute when it develops over 24 to 48 hours. Such patients are indeed at high risk for neurological complications, with mortality rates as high as 50% in some studies. Induction of rapid hyponatremia has similarly been shown to cause severe neurological dysfunction in rabbits, and virtually all animals so treated die with marked brain edema. In these animals, brain water content increased by an amount equivalent to the fall in their serum [Na^+], and brain electrolyte contents did not decrease significantly, indicating an absence of brain volume regulation (9).

Thus, when hypoosmolality develops at a rate that exceeds the brain's ability to regulate its volume by electrolyte losses, severe brain edema results, potentially leading to neurological dysfunction and sometimes death. It is therefore important to define the time course over which brain volume regulation can occur. This has been studied in rats by measuring brain water and electrolyte contents at various times after induction of an acute dilutional hyponatremia (10). Na^+ and Cl^- losses began very rapidly, generally within 30 minutes, whereas brain K^+ losses were somewhat more delayed. Nonetheless, all electrolyte losses were found to be maximal by 3 hours, and they completely accounted for the degree of brain volume regulation that was achieved over this period. Although brain edema still occurred, with measured increases in brain water from 6 to 9%, the ability of the brain to lose electrolytes rapidly within several hours limited the severity of brain swelling. These results are consistent with many experimental studies in animals that have reported variable neurological symptoms and survival rates following induction of acute hyponatremia, since over short periods of time (i.e., several hours) relatively small differences in the rates of loss of electrolytes can have profound effects on the resulting brain edema and neurological dysfunction.

2.2. Adaptation to Chronic Hypoosmolality

In contrast to acute hyponatremia, many experimental studies of chronic hyponatremia have been characterized by a relative absence of neurological symptoms and mortality. These findings suggest that more complete degrees of brain volume regulation occur after longer periods of sustained hyponatremia. Studies in rats in which hyponatremia was maintained for 21 days confirmed virtually complete normalization of brain water content (11). However, in these and other studies the measured electrolyte losses accounted for only 60 to 70% of the observed brain volume regulation, which suggested a potential contribution from losses of other brain solutes as well. Subsequent studies confirmed that brain content of most organic osmolytes also decreases markedly during induced hyponatremia in mice (12) and rats (13,14). The organic osmolytes involved in volume regulation are amino acids, methylamines, and polyols. The major organic osmolytes in the brain are glutamine, glutamate, taurine, and myoinositol. Organic osmolytes of lesser significance include several other amino acids, two methylamines glycerophosphorylcholine (GPC) and betaine], phosphocreatine/creatine, and the neurotransmitter γ-aminobutyric acid (GABA). *N*-acetylaspartate (NAA) is included among the solutes that are lost during volume regulation, but this amino acid represents a relatively minor component of the total brain solute losses (2). Figure 2 shows the relative brain losses of organic osmolytes compared to electrolytes after 14 days of sustained hyponatremia in rats (14). Total brain electrolyte losses are larger, as expected, nonetheless the measured brain organic osmolyte losses accounted for roughly one third of the measured brain solute losses during sustained hypoosmolality. Such coordinate losses of both electrolytes and organic osmolytes from brain tissue enable very effective regulation of brain volume during chronic hyponatremia (Fig. 1). Consequently, it is now clear that cellular volume regulation *in vivo* occurs predominantly through depletion, rather than intracellular osmotic "inactivation," of a variety of intracellular solutes (2). Studies using NMR spectroscopy in hyponatremic patients have confirmed that similar mechanisms occur in humans with hyponatremia (15).

In addition to physiological implications for brain volume regulation, the large decrease in brain organic osmolyte contents over relatively short periods (i.e. <48 hours) has potentially important functional implications. Such relatively rapid decreases suggest the possibility that some of these losses are occurring via effluxes of intracellular osmolytes from brain cells during the process of volume regulation. This could result in transiently increased local brain extracellular fluid concentrations of organic osmolytes, which in the case of amino acids could produce significant effects on neuronal membrane potential. In particular, given the known actions of glutamate as an excitatory neurotransmitter, locally increased brain glutamate concentrations occurring at the time of the active phase of volume regulation during hyponatremia could potentially account for some of the neurological abnormalities known to occur during this period, especially the increased incidence of seizure activity (16,17). This hypothesis could also explain, at least in part, the observation that when hypoosmolality is maintained for longer periods of time, both animals and patients become less symptomatic, since increased brain neurotransmitter concentrations would likely occur only transiently during the initial development of hyponatremia and then return to more normal levels after the completion of brain volume regulation.

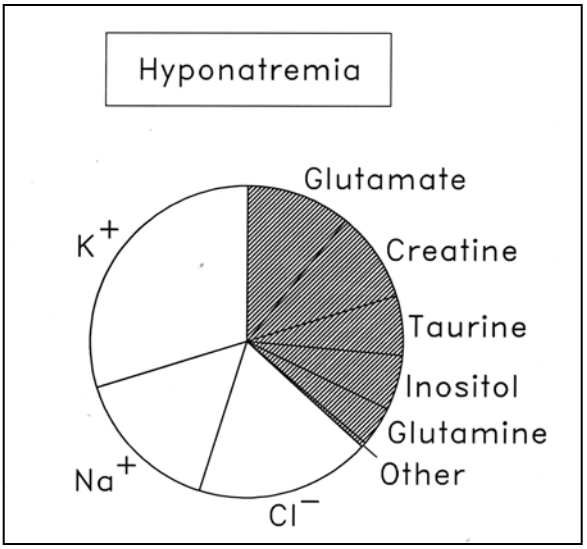

Figure 2. Relative decreases in individual brain electrolytes and organic osmolytes during adaptation to chronic (14 days) hyponatremia in rats. The category "other" represents GPC, urea, and several other amino acids. Reproduced with permission from (2).

2.3. Cellular Mechanisms Underlying Brain Adaptation to Hypoosmolality

Although brain volume regulation in response to perturbations of extracellular osmolality represents the most dramatic demonstration of volume regulation in response to changes in extracellular osmolality, the ability to regulate intracellular volume is an evolutionarily conserved mechanism inherent to variable degrees in most cells. Abundant *in vitro* experimentation has yielded important insights into the cellular mechanisms that underlie this important adaptive process (18).

With acute decreases in external osmolality, cells initially behave as osmometers and swell in proportion to the reduction in extracellular osmolality as a result of movement of water into the cells along osmotic gradients. Very soon thereafter, a process known as volume regulatory decrease (VRD) in cell volume begins, in which intracellular solutes are extruded together with osmotically obligated water (19). The time necessary to activate RVD and restore normal, or near-normal, cell volume is variable across different cell types. RVD occurs very rapidly *in vitro,* with a 70-80% recovery of normal cell volume reached within a few minutes in most brain and epithelial cells (19,20).

RVD has been studied in detail in astrocytes and neurons from primary cultures (20,21), in neuroblastoma (22) and glioma (23) cells lines. The osmolytes responsible for RVD are essentially the same in most cell types and can be grouped into two broad categories: electrolytes (predominantly K^+ and Cl^-) and organic osmolytes (amino acids, polyalcohols, sugars, and methylamines). In most cells examined to date, electrolyte fluxes appear to occur by diffusive pathways, i.e., K^+ and Cl^- efflux through separate volume-sensitive channels, and organic osmolytes through “leak pathways” with no

significant contribution from energy-dependent carriers (18). In brain cells, swelling activates at least two different types of K^+ channels, both a large and a small conductance channel (24). The volume-sensitive Cl^- channel (VSCC) has high selectivity of anions over cations, but exhibits broad anion selectivity, displaying permeability to the majority of monovalent anions (25,26). Although the molecular species of VSCC are as yet unidentified, recent evidence has supported the ClC3 channel gene as encoding the channel protein responsible for the volume-sensitive Cl^- current (27), but different types of VSCC and other anion-permeating molecules coincide in the same cell allowing for participation of more than one VSCC in RVD (28).

Although many different organic osmolytes are also released by cells during RVD, their efflux pathways have been characterized for only a few, particularly taurine and myoinositol. In general, these are bidirectional leak pathways with net solute movement depending on concentration gradient direction (18,29). Organic osmolyte pathways commonly exhibit a pharmacologic profile similar to that of the VSCC, suggestive of a common pathway with Cl^-, or of a close connection between the two pathways (29,30). Other amino acids also responsive to swelling are glycine, GABA, glutamate, and aspartate, which contribute to correction of osmotic disturbance. Recent evidence of hyposmolality-induced glutamate release that is insensitive to Cl^- channel blockers is different from the pattern found with most other organic osmolytes (17). This suggests either different pathways, or different stimuli and mechanisms for release, of this amino acid.

Exactly how cells sense volume changes is a critical step in the reactions activated to achieve volume correction. Among possible mechanisms considered to play this role are membrane receptors such as integrins or receptors with intrinsic tyrosine kinase activity, cytoskeleton rearrangements, dilution of cytosolic macromolecules, decrease in intracellular ionic strength, stretch-induced activation of adhesion molecules, activation of phospholipases, or changes in the concentration of signaling molecules such as calcium or magnesium (31). Calcium and protein kinases are among the most likely candidates to act as osmotransductory elements. One of the most constant features of hyposmolar swelling is an increase in systolic Ca^{++}. Despite this, the main corrective osmolyte efflux pathways and consequently RVD are Ca^{++}-independent in a large variety of cell types. This is the case for brain cells, in which VSCC, VSKC, and organic efflux pathways are largely Ca^{++}-independent (24). This is an area of active ongoing research, and the reader is referred to excellent recent reviews of this topic for more details (18,31,32).

2.4. Recovery from Hypoosmolality (Deadaptation)

Compensatory adaptations that enable organisms to survive chronic perturbations of body homeostasis must be reversed after recovering from the underlying abnormality. In some cases reversal of the adaptive process, or "de-adaptation," may be more problematical than the initial adaptation itself. This appears to be true for correction of chronic hyponatremia (33). Multiple studies have shown that rapid correction of chronic hyponatremia causes dehydration of brain tissue (34-36), and in some cases demyelination of white matter in various parts of the brain (37-39). Because this dehydration occurs to a greater degree in hyponatremic rats than in normonatremic rats following similarly large increases in osmolality, it has been suggested that this phenomenon reflects a loss of osmotic buffering capacity by brain tissue as a

consequence of the initial brain solute losses that allowed survival despite hypoosmolar conditions (33).

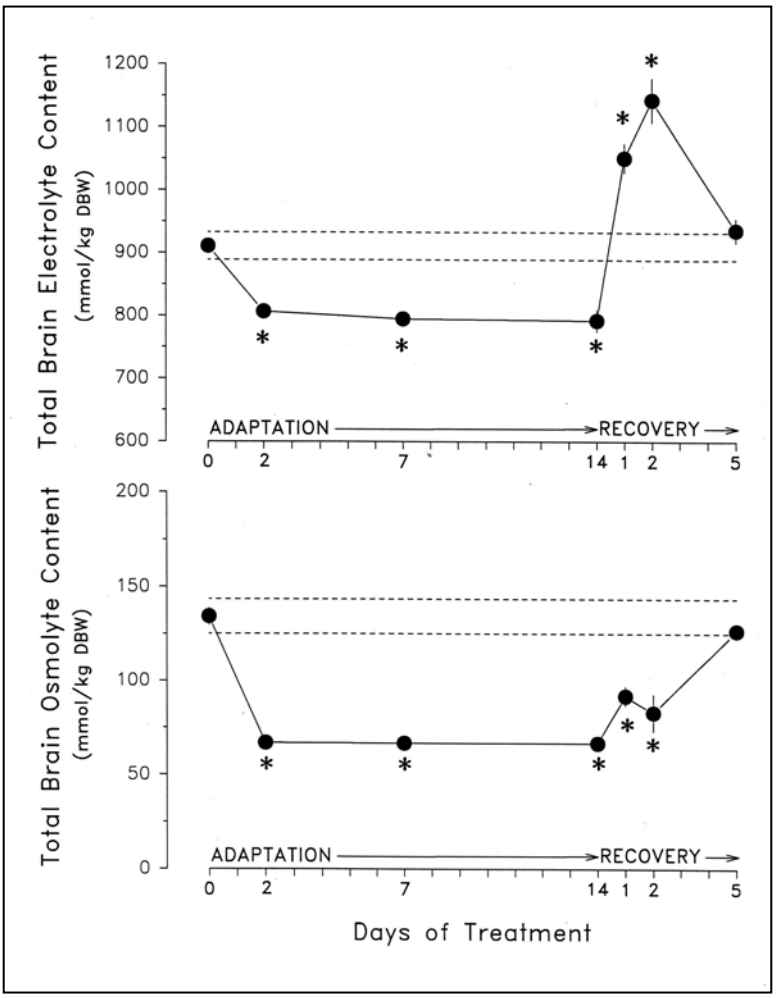

Figure 3. Time course of changes in brain electrolytes (top panel) and organic osmolytes (bottom panel) during adaptation to chronic hyponatremia and following correction of hyponatremia in rats. Dotted lines depict normal brain levels; *p<0.05 compared to day 0. Reproduced with permission from (3).

Several studies have now demonstrated markedly different rates of reaccumulation of brain solutes after normalization of hypoosmolality in hyponatremic mice and rats (13,40). In response to hyponatremia, brain tissue rapidly loses all classes of osmotically active solutes, including both electrolytes and organic osmolytes, thereby allowing the brain to efficiently regulate its volume. On the other hand, after recovery from

hyponatremia organic osmolytes, with the exception of glutamate, return to normal brain contents very slowly over a period of many days, while electrolytes reach normal or supranormal contents in the brain within 24 hours after correction of hyponatremia (Fig. 3). This slow reaccumulation of organic solutes is very analogous to the similarly slow increases in osmolytes that occur during chronic hypernatremia (see below), and suggests that in general the brain is much better able to lose organic solutes than to reaccumulate them. Furthermore, the rapid electrolyte reaccumulation after correction of hypoosmolality consists mainly of the extracellular electrolytes Na^+ and Cl^-, and these significantly overshoot brain contents necessary to achieve normal volume regulation (Fig. 3). This again is quite analogous to the rapid increases in brain Na^+ and Cl^- that occur in response to acute hyperosmolality (41,42), and it suggests that in this situation these electrolytes are similarly gaining access to the brain rapidly via the CSF and are acting to stabilize intracellular volume (43). Consequently, the mechanisms that enable the brain to adapt to hypoosmolar conditions and those that accomplish de-adaptation after subsequent normalization of plasma osmolality are not simply mirror images of each other.

Knowledge of this greater inefficiency of brain solute reaccumulation and volume regulation following correction of chronic hyponatremia is very relevant to understanding the pathological sequelae known to be associated with rapid correction of chronic hypoosmolality, namely the occurrence of osmotic demyelination. Every adaptation made by the body in response to a perturbation of homeostasis bears within it the potential to create new problems. Although the mechanism(s) by which rapid correction of hyponatremia leads to brain demyelination remain unproven, this pathological disorder likely results from the brain dehydration that has been demonstrated to occur following correction of plasma $[Na^+]$ toward normal ranges in animal models of chronic hyponatremia. Because the degree of osmotic brain shrinkage is greater in animals that are chronically hyponatremic than in normonatremic animals undergoing similar increases in plasma osmolality, by analogy the brains of human patients adapted to hyponatremia are likely to be particularly susceptible to dehydration following subsequent increases in osmolality. This, in turn, can lead to pathological demyelination. Further support for dehydration-induced demyelination has come from recent reports that acute hyperosmolality can also cause demyelination in experimental animals (44), though larger increases in plasma osmolality are required than in hyponatremic rats. Although the exact mechanisms responsible for production of brain demyelination following correction of hyponatremia remain uncertain, one possibility is that acute brain dehydration produced by rapid correction could potentially disrupt the tight junctions of the blood-brain barrier. Recent magnetic resonance studies in animals have shown that chronic hypoosmolality predisposes rats to opening of the blood-brain barrier following rapid correction of hyponatremia, and that the disruption of the blood-brain barrier is highly correlated with subsequent demyelination (45). A potential mechanism by which blood-brain barrier disruption might lead to subsequent myelinolysis is via an influx of complement, which is toxic to the oligodendrocytes that manufacture and maintain myelin sheaths of neurons, into the brain (46).

3. HYPEROSMOLALITY

Hyperosmolality and hypernatremia usually occurs as a result of hypotonic fluid losses that are not compensated by sufficient water intake to maintain body fluid homeostasis. Less commonly, excess NaCl ingestion or administration can cause hyperosmolality as a result of solute excess. Although hyperosmolality can develop in association with a broad spectrum of disease processes in people of all ages, infants and elderly individuals are particularly susceptible (4). The neurological symptoms of hyperosmolar states are a result of the cellular dehydration produced by osmotic shifts of water from the intracellular fluid space to the more hypertonic extracellular fluid space. The clinical symptomatology is related both to the severity of the hyperosmolality and also to the rate at which it develops (47). The symptoms of hyperosmolar states are a consequence of neurological dysfunction resulting from cellular dehydration; these include irritability, restlessness, stupor, muscular twitching, hyperreflexia, spasticity, and in severe cases, seizures, coma, and ultimately death (4).

Because cell membranes are relatively more permeable to water than to electrolytes, a rapid increase in plasma osmolality causes the brain to shrink. The brain subsequently undergoes an adaptation process involving the accumulation of solutes to restore the brain volume to its normal level. This adaptation process involves rapid accumulation of inorganic ions and slower accumulation of organic osmolytes, traditionally termed "idiogenic osmoles" (48). Marked differences in symptoms, and hence in recovery from hyperosmolality, exist because of the time-dependent nature of this complex brain adaptation. Optimal treatment of hyperosmolar patients is facilitated by knowledge of the basic mechanisms underlying the process of adaptation to the hyperosmolar state. Just as with hypoosmolality, neurological symptoms and mortality are generally higher in patients with acute rather than chronic hypernatremia. Consequently, it is useful to consider brain adaptation to these different pathological states separately.

3.1. Adaptation to Acute Hyperosmolality

Acute hypernatremia, generally defined as the development of serum $[Na^+]$ >145 mmol/L in 24 to 48 hours, is relatively uncommon. It can, however, be seen in infants as a result of accidental salt poisoning or severe gastroenteritis. It occurs less commonly in adults, although patients with untreated diabetes insipidus who are unable to drink can develop severe hypernatremia very rapidly. Despite the relative rarity of acute hyperosmolality, it is important to understand the pathophysiology underlying the neurological symptoms associated with this disorder because of its marked morbidity and mortality, which can be as high as 75% in adults and 45% in children (4,47).

Acute hypernatremia is typically induced in animals by intraperitoneal injections of hypertonic NaCI, which causes a prompt reduction in brain water content. However, the rapid loss of brain water is less than would be expected if the brain behaved as a perfect osmometer, because the brain is capable of rapidly accumulating solute to stabilize its volume. In a study using rats, three hours of hypernatremia (serum $[Na^+]$ >200 mmol/liter) decreased brain water by 14% and promoted increases in contents of brain Na^+ and Cl^- of 34% and 60%, respectively; K^+ content was unaltered (8). Other studies of acute (15-120 min) hypernatremia in rats showed that the reduction in brain volume was proportional to the increase in plasma osmolality and generally stabilized by 15 to 30 minutes after the NaCI injection. However, by longer periods, i.e., 30 and 120 minutes,

the brain water loss was only 35% of that predicted, which indicates that significant volume regulation had already occurred (49,50). This acute but partial volume regulation was due to rapid increases in tissue electrolytes. The accumulation of Na^+ and Cl^- was attributed to influx from the CSF, whereas the slower rise in tissue K^+ content was related to an influx from plasma across the blood-brain barrier.

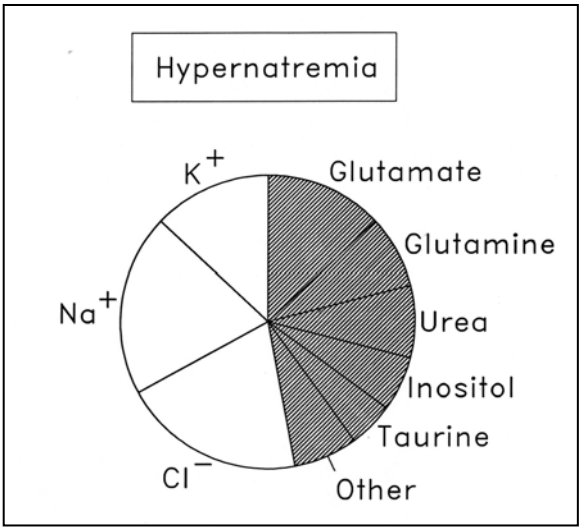

Figure 4. Relative increases in individual brain electrolytes and organic osmolytes during adaptation to chronic hypernatremia in rats. The category "other" represents GPC, urea, and several other amino acids. Reproduced with permission from (2).

A central problem in studies of brain volume regulation has been an inability to distinguish the changes that occur in the intracellular versus the extracellular spaces. A seminal study by Cserr and coworkers used ion-selective electrodes to resolve this issue (43). Rats given a single intraperitoneal injection of NaCl experienced a 7% loss of brain water after 30-90 minutes, but this loss was related entirely to a decrease in and extracellular water content; intracellular water content remained at basal levels. Estimates of intracellular and extracellular ion contents indicated that extracellular Na^+, Cl^-, K^+ decreased by 32, 21, and 42%, respectively, whereas intracellular contents of these ions increased by 100, 169, and 5%, respectively. In contrast, studies of organic osmolytes have indicated that acute hypernatremia is not associated with increases in organic solutes that are sufficient to appreciably contribute to brain volume regulation (41,48). Thus, acute hypernatremia is characterized by a rapid loss of total brain water, but protection of intracellular volume by an almost equivalently rapid accumulation of electrolytes from the extracellular fluid, CSF, and plasma.

3.2. Adaptation to Chronic Hyperosmolality

In most hypernatremic patients, hyperosmolality develops gradually over a period of several days regardless of the etiology (47). Although morbidity and mortality rates are reported to be high in both adults and children, interpretation of these findings is difficult because death is often a result of the underlying disease that caused the fluid imbalance (4). Nonetheless, chronic hypernatremia is generally better tolerated with less neurological symptomatology than occurs during acute hypernatremia of comparable magnitude, which indicates that the brain is able to adapt to hyperosmolar conditions over longer periods of time. This has been attributed to a slower accumulation of organic solutes by the brain, and recent studies have provided greater insights into this adaptation process.

In animals, when hypernatremia persists beyond several days, total brain tissue water content slowly returns to normal levels (8,41). This restoration of total brain water does not result from continued accumulation of electrolytes but rather from accumulation of specific organic osmolytes (41,42,48,51). The organic osmolyte accumulation generally accounts for 30-50% of the solute accumulation in hypernatremic animals. The organic osmolytes involved with volume regulation to hyperosmolar conditions are essentially the same substances found to be involved with adaptation to chronic hypoosmolality, as described earlier. No single study has quantified the major electrolyte and organic osmolyte changes in the brains of chronically hypernatremic animals. However, using data from several sources, one can estimate the relative contributions of major osmolytes during adaptation to chronic hypernatremia (2) (Fig. 4). As for adaptation to hypoosmolality, NAA is included among the solutes that accumulate during volume regulation in response to hyperosmolality, but this amino acid represents a relatively minor component of the total brain solute losses; studies of in rats indicated that NAA accounted for only 16% of the total increase in brain amino acids after chronic salt loading (42).

Organic osmolytes accumulate relatively slowly in brain following induction of hypernatremia. Indirect measurements of the brain contents of undetermined solutes (i.e., total osmolality minus the sum of tissue electrolytes) indicated that organic osmolytes begin to accumulate after 9 to 24 hours but do not reach a steady-state level until 2 to 7 days (52). *In vitro* studies of cultured brain cells have corroborated this delayed and slow rate of organic osmolytes accumulation.

3.3. Cellular Mechanisms Underlying Brain Adaptation to Hyperosmolality

With acute increases in external osmolality, cells initially behave as osmometers and shrink in proportion to the reduction in extracellular osmolality as a result of movement of water out of cells along osmotic gradients. Soon thereafter, a process known as *volume regulatory increase (VRI)* in cell volume begins, in which cells accumulate intracellular solutes together with osmotically obligated water (19). Similar to VRD, the time necessary to activate RVI and restore normal, or near-normal, cell volume is variable across different cell types. However, in general RVI occurs more slowly than VRD in most cell types where this has been carefully studied.

The mechanisms responsible for brain and cell organic osmolytes accumulation during hyperosmolar conditions remain inadequately understood, but likely represent a combination of cellular uptake (e.g., myoinositol and taurine (53)) and synthetic

mechanisms (e.g., glutamate), as has been well described in renal medulla where hypertonic conditions predominate (54). Circulating blood levels of many amino acids are increased during hypernatremia, and these may serve as a precursor pool for brain osmolytes (48). One important issue that remains to be resolved is the intracellular/extracellular distribution of organic and inorganic osmolytes in the brain during chronic hyperosmolality. Studies of other systems would suggest that organic osmolytes are preferentially accumulated intracellularly and thus replace the inorganic solutes, which are primarily responsible for the acute phase of brain cell volume regulation.

3.4. Recovery from Hyperosmolality (Deadaptation)

Accumulation of solutes enables the brain to adapt to hyperosmolar states, and thus is life saving, but during correction of the hyperosmolality this increase in total brain solute content can lead to neurological dysfunction due to osmotic shifts of water into the now more hypertonic intracellular fluid space. As with the adaptation process, both the duration of the hyperosmolality and the rate of the correction determine the degree of brain of edema that occurs. When acute hyperosmolar animals are given access to water, they recover relatively rapidly. The recovery phenomenon involves a transient but small increase in tissue water and a relatively rapid loss of the electrolytes that accumulated during the hyperosmolar episode. In contrast, during recovery from chronic hypernatremia, restoration of brain organic solute contents to normal levels occurs slowly over 24 to 48 hours. Studies in rats found that betaine fell to normal levels within 24 hours whereas glutamine, glutamate, taurine, phosphocreatine, and GPC took two days to achieve normal levels, and myoinositol remained significantly elevated even after two days (41). The mechanisms responsible for organic and inorganic solute loss during recovery from hypernatremia are not known, but are likely similar to those responsible for VRD during adaptation to hypoosmolality.

This slow dissipation of accumulated organic solutes is the basis for clinical recommendations that chronic hypernatremia be corrected relatively slowly over 48 hours (4,47). In support of a slow correction, studies of children have reported a high incidence of seizures following rapid correction of severe hypernatremia, presumably caused by brain edema (55,56). Although well-controlled studies of optimal correction rates in adults do not exist, based on what is known about the rates of organic solute losses from brain tissue in animals it seems prudent to continue to recommend the more cautious approach of prompt but gradual correction of chronic hypernatremia and hyperosmolality (57).

4. SUMMARY

Multiple studies over several decades have provided evidence that both electrolytes and organic osmolytes play crucial roles in regulating brain volume, both during increases as well as during decreases in extracellular fluid osmolality. In both situations, however, changes in brain electrolyte contents appear to occur more rapidly, and represent the first line of defense of brain volume during acute perturbations of body fluid tonicity, while organic osmolytes allow adaptation to more chronic perturbations. For both hyperosmolality and hypoosmolality, the rate of development of the disorder is an

important determinant of neurological morbidity and mortality, since sufficiently rapid changes in tonicity can exceed the brain's capacity to regulate its volume leading to more severe degrees of brain edema or dehydration.

Recovery from both hyper- and hypoosmolality requires reversal of the adaptive processes that enabled regulation of brain volume in response to the initial insult. However, adaptation and recovery are not symmetrical processes. Marked differences occur in the speed with which the brain is able to lose or to reaccumulate different types of solutes after recovery from chronic disturbances of body fluid tonicity. In general, accumulation, or reaccumulation, of organic solutes by brain tissue is a much slower process than volume regulatory losses of such solutes. As with the adaptation process, the rate of recovery is an important determinant of subsequent morbidity and mortality, since rapid corrections of osmolality can also exceed the capacity of the brain to readjust its solute content, and consequently its volume, back to normal levels.

Whether or not transient excesses or deficiencies of either electrolytes or specific organic osmolytes in brain intracellular or extracellular fluid contribute to functional disturbances independently of changes in brain volume is an intriguing question that has not been sufficiently evaluated. Also remaining to be answered are questions regarding other physiological, pathophysiological, and pharmacological factors that either impair or enhance volume regulatory processes, and thereby modify the neurological manifestations accompanying disorders of body fluid osmolality in humans. Finally, a complete understanding of the cellular mechanisms underlying adaptation to and deadaptation from acute and chronic perturbations of body osmolality will be essential to design the most enlightened, and therefore appropriate, treatments for these disorders.

5. QUESTION AND ANSWER SESSION

SESSION CO-CHAIR NAMBOODIRI: Thank you, Dr. Verbalis. Now we will have a few questions.

DR. PHILIPPART: Philippart, Los Angeles. I wonder if you also look at plasma bicarbonate.

DR. VERBALIS: Plasma bicarbonate is basically unchanged, as it is in humans with hyponatremia. It doesn't appear to be a major factor in this disorder.

SESSION CO-CHAIR KOLODNY: Brian Ross?

DR. ROSS: First is just a comment. I presume since we can measure all of these osmolytes with MR spectroscopy in the human brain, that you now routinely use spectroscopy in your patients.

DR. VERBALIS: You presume wrong, but it has been done. The same kind of decreases in osmolytes shown in the animal models has also been documented in human brains at UCLA using magnetic resonance spectroscopy. But it's not being used routinely for therapeutic purposes at this time.

DR. ROSS: I am challenging you to do so since you see these patients. But, my next point is a comment which I discussed briefly with you earlier and then hearing Dr. Baslow's presentation, you note that creatine is a major osmolyte in the brain.

If creatine is truly a major osmolyte and fluctuates with this huge 15-20 percent decrease with hypoosmolality, how do we explain the maintenance of energy metabolism because creatine and phosphocreatine are one and the same thing in your hands? And in Dr. Baslow's theory, he needs to demonstrate changes in energy metabolism.

DR. VERBALIS: I actually went one step further than that. I showed you my rats playing basketball, despite a 30 percent decrease in brain glutamate in those rats.

DR. ROSS: I understand.

DR. VERBALIS: So, how can a rat have normal motor transmission and normal motor function with a 30 percent decrease in a major excitatory amino acid in addition to a 20% decrease in creatine?

DR. ROSS: Right.

DR. VERBALIS: And I think that I gave you my explanation to both of those points, which is I believe that one of the factors that has not been taken into account in understanding brain energy metabolism, or even neurotransmission, are the separate pools of these various osmolytes that are involved with distinct cellular processes.

So, I believe that creatine, glutamate, aspartate, and NAA all have an osmoregulatory role which is separate from their other cellular roles, both metabolic and neurotransmission, and that it is possible to decrease one of these pools without necessarily impacting on the functions of the other pools.

That's the only way, for example, that I could explain normal motor function of these animals with those kinds of decreases in amino acids that we think are crucial for normal neural transmission, certainly in motor neurons but probably in all types of neurons for that matter. It's the only way one could understand it.

DR. ROSS: I had one more point to make. In all seriousness, I think you're absolutely right, of course, but we still do have the problems of these equilibria.

The proposal that was made - not in recent years, and this may have, therefore, not been brought to any of our attention, was actually made, in part, by R. L. Veech from 1979 or '80, in which he combines the Nernst equation, the Gibbs equation, and the osmotic equilibrium, the Donnan equilibrium, into one huge equation, saying that, actually, all of these things are linked.

So I think that there is a way in which Dr. Baslow's hypothesis and yours could really be saying the same thing.

DR. VERBALIS: Certainly, the water movement in association with the movement of both ions and organic particles across cell membranes is potentially very large. And so I am not by any means saying that I don't believe that inhibition of one part of that system might not result in what would be predicted, which is retention of water by the cell.

What I'm saying, however, is that based on our studies in this field, the brain has many other systems that are able to compensate for deficiencies, or excesses, of individual osmolytes.

So I'm not saying that that excess NAA might not produce exactly the effects that Dr. Baslow, and others, are postulating. What I am questioning is, why wouldn’t the same kinds of mechanisms that allow brains to adapt to an even more severe osmotic stresses allow them to regulate their volume in this situation as well? Why wouldn’t the same mechanisms come into play in a situation in which one molecular water pump was paralyzed, nonexistent, or somehow impaired? Why wouldn’t these other osmolytes be able to regulate the volume of these cells with increased water retention?

That's where I have trouble fitting the molecular water pump hypothesis together as a viable single hypothesis to explain the observations in Canavan disease.

PARTICIPANT: Sir, there is also hypernatremia. So can you give an explanation for that in this way?

DR. VERBALIS: Yes, you're right that severe hypernatremia has also produced pontine and extrapontine myelinolysis. And the answer is that hypernatremia is also an osmotic stress. If you take an animal, or a human, from a normal plasma osmolality up to 360 or 370 mOsm/kg H_2O, then the blood brain barrier will be disrupted; complement and other immune proteins can then gain access into the brain and potentially can produce the same kind of demyelinating syndrome.

The difference between correction of hyponatremia and induction of hypernatremia is that in the chronic hyponatremic state, the brain has lost its pool of excess osmolytes, its osmotic "buffering capacity", so it's more susceptible to shrinkage and breaking the blood brain barrier with a lesser increase in plasma osmolality.

But if you take a normal human or animal up high enough in plasma osmolality, yes, you would break the blood brain barrier as well. If the increased osmolality is prolonged and sufficient, then the same pathophysiology as is seen with rapid correction of hyponatremia can and does occur.

The fact that this does occur is further proof for the blood-brain barrier disruption mechanism underlying the pathogenesis of pontine and extrapontine myelinolysis.

DR. COYLE: I just want to say that I concur with your suggestion that there are probably at least two pools of glutamate. The lesion studies indicate that probably less than ten percent of the total glutamate is in the neurotransmitter pool and the other ninety percent is in another pool. I'm not surprised that there's still a slam-dunk even when you have thirty percent of the brain glutamate decreased.

DR. VERBALIS: But would you expect, at least over prolonged periods of time, that this might, at least in some ways, impact upon the transmitter pool? Again, we don't see any evidence for that, not that we have measured those pools separately, nor would I know how to at this point. But it is impressive that, even for long periods of time, with severe depletion of these excitatory amino acids from the brain, there still is no apparent functional neurological effect of that dramatic phenomenon.

But, if I'm right and you're right, then just separate pools could explain that and allow one component to be markedly decreased without really impacting too severely on the others. If this is true, then it would imply preferential shunting into the neurotransmitter pool at the expense of the free cytosolic pool, which comprises the osmotically active component of cells.

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