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REVIEW

BRAIN VOLUME REGULATION IN RESPONSE TO CHANGES IN OSMOLALITY

J. G. VERBALIS*

Department of Medicine, 232 Building D, Georgetown University Medical Center, 4000 Reservoir Road NW, Washington, DC 20007, USA

Abstract—Hypoosmolality and hyperosmolality are relatively common clinical problems. Many different factors contribute to the substantial morbidity and mortality known to occur during states of altered osmotic homeostasis. The brain is particularly vulnerable to disturbances of body fluid osmolality. The most serious complications are associated with pathological changes in brain volume: brain edema during hypoosmolar states and brain dehydration during hyperosmolar states. Studies in animals have elucidated many of the mechanisms involved with brain adaptation to osmotic stresses, and indicate that it is a complex process involving transient changes in water content and sustained changes in electrolyte and organic osmolyte contents. Appreciation of the nature of the adaptation process, and conversely the deadaptation processes that occur after recovery from hypoosmolality and hyperosmolality, enables a better understanding of the marked variations in neurological sequelae that characterize hyperosmolar and hypoosmolar states, and provides a basis for more rational therapies.

Key words: hypernatremia, hyponatremia, osmolality, osmolytes, vasopressin, volume regulation.

Contents

*Corresponding author. Tel: +1-202-687-2818; fax: +1-202-687-2040.
E-mail address: verbalis@georgetown.edu (J. G. Verbalis).
Abbreviations: OVLT, organum vasculosum of the lamina terminalis; TRPV, transient receptor potential vanilloid; VRD, volume regulatory decrease; VSCC, volume-sensitive Cl^- channel.

Hypoosmolality and hyperosmolality are relatively common clinical problems (Verbalis, 2001). 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 pathologic changes in brain volume: brain edema during hypoosmolar states and brain dehydration during hyperosmolar states. This review 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 article the terms hyponatremia and hypoosmolality are used interchangeably, as are hypernatremia and hyperosmolality.

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* (Arieff, 1984; Fraser and Arieff, 1997), but the incidence and severity of such symptoms in hyponatremic patients are quite variable (Sterns, 1987). It is not unusual to find patients with low serum sodium concentrations ($[Na^+]$) who are relatively asymptomatic, whereas 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 its volume in response to hypoosmolality has been crucial to understanding this sometimes perplexing spectrum of clinical presentations of hypoosmolar patients.

Brain adaptation to hypoosmolality

Experimental studies in animals over the last century have elucidated many of the physiological mechanisms underlying brain adaptation to hypoosmolality (Arieff et al., 1976; Holliday et al., 1968; Yannet, 1940). After decreases in plasma osmolality, water moves into the brain along osmotic gradients, causing cerebral edema. In response, the brain loses solute from the extracellular (Melton et al.,

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1987) and the intracellular (Holliday et al., 1968; Yannet, 1940) 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 severe neurological symptoms associated with hyponatremia are thought to reflect brain edema as a consequence of osmotic water movement into the brain (Fraser and Arieff, 1997). However, after 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 seemingly 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 brain 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.

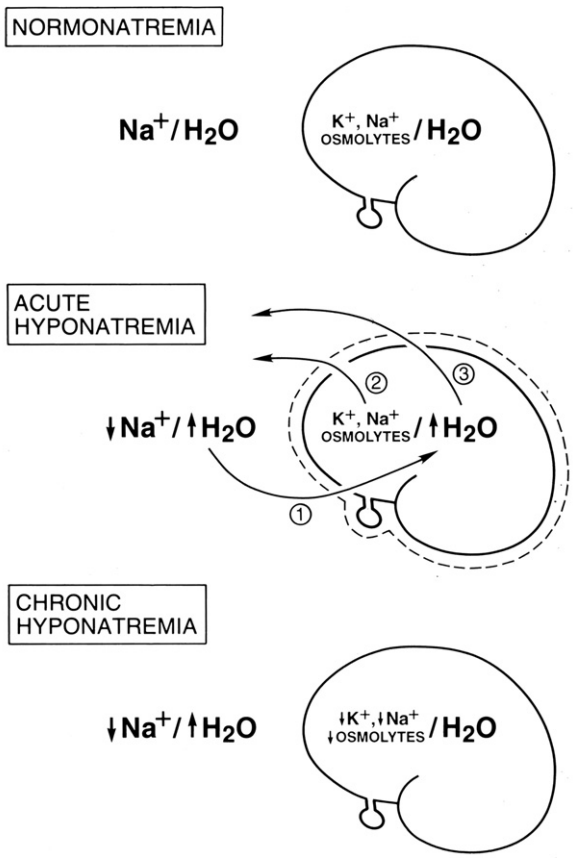

Fig. 1. Schematic diagram of brain volume adaptation to hyponatremia. Under normal conditions, brain osmolality and extracellular fluid (ECF) osmolality are in equilibrium (top panel, for simplicity the predominant intracellular solutes are depicted as K^+ and organic osmolytes, and the extracellular solute as Na^+). After 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 (Verbalis, 2001).

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 24–48 h. 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 (Arieff et al., 1976).

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 (Melton et al., 1987). Na^+ and Cl^- losses began very rapidly, generally within 30 min, whereas brain K^+ losses were somewhat more delayed. Nonetheless, all electrolyte losses were found to be maximal by 3 h, 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 after induction of acute hyponatremia because over short periods (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.

Adaptation to chronic hypoosmolality. In contrast to acute hyponatremia, many experimental studies of chronic hyponatremia have been characterized by a relative absence of severe 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 (Verbalis and Drutarosky, 1988). However, in these and other studies, the measured electrolyte losses accounted for only 60%–

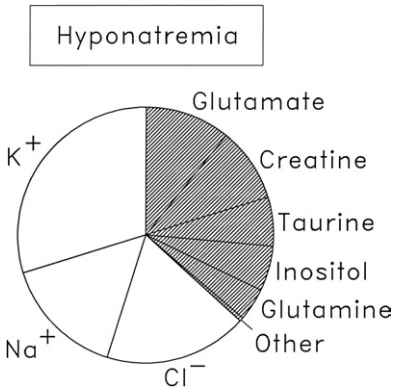

Fig. 2. Relative decreases in individual brain electrolytes and organic osmolytes during adaptation to chronic (14 days) hyponatremia in rats. The category "other" represents GPC, urea, and several other amino acids. Reproduced with permission from (Gullans and Verbalis, 1993).

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 (Thurston et al., 1987) and rats (Lien et al., 1991; Verbalis and Gullans, 1991). Fig. 2 shows the relative brain losses of organic osmolytes compared to electrolytes after 14 days of sustained hyponatremia in rats (Verbalis and Gullans, 1991). Total brain electrolyte losses are larger, as expected; nonetheless, the measured brain organic osmolyte losses accounted for approximately 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 (Gullans and Verbalis, 1993). Studies using NMR spectroscopy in hyponatremic patients have confirmed that similar mechanisms occur in humans with hyponatremia (Videen et al., 1995).

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 (Pasantes-Morales et al., 2002b).

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 (Grantham, 1977). The time necessary to activate VRD and restore normal, or near-normal, cell volume is variable across different cell types. VRD 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 (Grantham, 1977; Pasantes-Morales et al., 1993).

VRD has been studied in detail in astrocytes and neurons from primary cultures (Pasantes-Morales et al., 1993; Sanchez-Olea et al., 1993), in neuroblastoma (Basavappa et al., 1996) and glioma (Strange and Morrison, 1992) cells lines. The osmolytes responsible for VRD 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, that is, K^+ and Cl^- efflux through separate volume-sensitive channels, and organic osmolytes through "leak pathways," with no significant contribution from energy-dependent carriers (Pasantes-Morales et al., 2002b). In brain cells, swelling activates at least two different types of K^+ channels, both a large and a small conductance channel (Pasantes-Morales and Morales, 2000). 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 (Nilius et al., 1997; Okada, 1997). 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 (Hermoso et al., 2002), but different types of VSCC and other anion-permeating molecules coincide in the same cell allowing for participation of more than one VSCC in VRD (Sardini et al., 2003).

Although many different organic osmolytes are also released by cells during VRD, their efflux pathways have been characterized for only a few, particularly taurine and myo-inositol. In general, these are bidirectional leak pathways, with net solute movement depending on concentration gradient direction (Kirk, 1997; Pasantes-Morales et al., 2002b). Organic osmolyte pathways commonly exhibit a biological profile similar to that of the VSCC, suggestive of a common pathway with Cl^-, or of a close connection between the two pathways (Kirk, 1997; Pasantes-Morales, 1996). Other amino acids also responsive to swelling are glycine, GABA, glutamate, and aspartate, which contribute to correction of osmotic disturbance. Recent evidence of hypoosmolality-induced glutamate release that is insensitive to Cl^- channel blockers is different from the pattern found with most other organic osmolytes (Pasantes-Morales et al., 2002a). 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 for all of the mechanisms activated to achieve volume correction. Some of the most exciting new data have come from studies of brain osmoreceptors (Verbalis, 2007). "Ef-

fective" solutes are those that penetrate cells slowly, or not at all, thereby creating an osmotic gradient that causes an efflux of water from osmoreceptor cells. The resultant shrinkage of osmosensitive neurons has been found to activate membrane non-selective cationic conductances that cause generation of an inward current; if of sufficient magnitude, the resulting depolarization of the osmoreceptor neuron then generates an action potential (Bourque et al., 2002). Conversely, "ineffective" solutes that penetrate cells readily create no osmotic gradient, and thus have little to no effect on the cell volume of the osmoreceptors. Electrophysiological studies of neurons in the *organum vasculosum of the lamina terminalis* (OVLT) show that they display changes in action potential firing rate that vary in proportion to the tonicity of extracellular fluid, supporting the likelihood that these cells represent osmosensory neurons (Bourque et al., 2007). Osmotically-evoked changes in the firing rate of the OVLT neurons, in turn, synaptically regulate the electrical activity of downstream effector neurons, importantly including the magnocellular AVP neurons in the hypothalamus, through graded changes in release of the excitatory neurotransmitter glutamate. This mechanism agrees well with the observed relationship between the effect of specific solutes such as sodium, mannitol, and glucose on arginine vasopressin (AVP) secretion.

The cellular osmosensing mechanism used by the OVLT cells is an intrinsic depolarizing receptor potential, which these cells generate through a molecular transduction complex. Recent results suggest that this likely includes members of the *transient receptor potential vanilloid (TRPV)* family of cation channel proteins. These channels are generally activated by cell membrane stretch to cause a non-selective conductance of cations, with a preference for Ca^{2+}. Multiple studies have characterized various members of the TRPV family as cellular mechanoreceptors in different tissues (Liedtke and Kim, 2005). Both *in vitro* and *in vivo* studies of the TRPV family of cation channel proteins has provided evidence supporting roles for TRPV1, TRPV2, and TRPV4 proteins in the transduction of osmotic stimuli in mammals (Liedtke, 2007). An N-terminal *trp1* variant has been found to be expressed in OVLT cells, and *trp1* knock-out mice have been found to have defects in osmotically-stimulated AVP secretion and thirst (Bourque et al., 2007). Heterologous expression of *trpv2* in CHO cells causes an activation of Ca^{2+} influx in response to hypotonicity, a response that can be mimicked by cell membrane stretch (Liedtke, 2007). *trpv4*-Transfected cells respond similarly to hypotonicity and mechanical stretch, and display deficient VRD in response to hypoosmolality (Becker et al., 2005). But *in vivo* studies have yielded inconsistent findings. *trpv4* Knock-out mice were found to have a potentiated AVP response to a combined hypertonic and hypovolemic stimulus in one study (Mizuno et al., 2003), but blunted responses of both AVP secretion and thirst to a selective hypertonic stimulus in another (Liedtke and Friedman, 2003). These findings are not necessarily contradictory, as both AVP secretion and thirst are likely under bimodal control, that is, they are stimulated by hypertonicity and inhibited by hypotonicity (Verbalis, 1993). In support of this possibility, treatment with desmopressin led to hyponatremia in *trpv4* knock-out mice but not wild-type controls, indicating a failure of osmotic inhibition of drinking (Liedtke and Friedman, 2003). Thus, different channels and/or different sets of osmoreceptor cells may mediate opposite responses to cell membrane stretch, although osmosensitive inhibitory neurons have not yet been identified in the OVLT (Bourque et al., 2007).

The combined studies to date, therefore, strongly support the characterization of TRPV1, TRPV2, and TRPV4 as "osmo-mechano-TRPs" that are important for sensing cell volume (Liedtke, 2007). Although the details of exactly how and where various members of the TRPV family of cation channel proteins participate in osmoregulation in different species remain to be ascertained by additional studies, a strong case can already be made for their involvement in the transduction of osmotic stimuli in the neural cells in the OVLT and surrounding hypothalamus that regulate osmotic homeostasis, which appears to have been be highly conserved throughout evolution (Liedtke, 2007). Future studies will be necessary to address still unanswered questions, including: the exact structure of the molecular transduction complex that regulates the opening of cationic channel(s) in response to changes in tonicity; whether different heteromultimeric combinations of TRPV1, TRPV2, and TRPV4, and possibly other cationic channels, mediate differential responses to changes in tonicity; whether separate excitatory and inhibitory osmoreceptors control AVP secretion and thirst; and their relation to other mechanisms potentially involved in osmoreception including 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 (Hoffmann, 2000).

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 "deadaptation," may be more problematical than the initial adaptation itself. This appears to be true for correction of chronic hyponatremia (Verbalis, 1998). Multiple studies have shown that rapid correction of chronic hyponatremia causes dehydration of brain tissue (Adler et al., 1994; Berl, 1990; Sterns et al., 1989), and in some cases demyelination of white matter in various parts of the brain (Kleinschmidt-DeMasters and Norenberg, 1981; Laureno, 1980; Sterns et al., 1986). Because this dehydration occurs to a greater degree in hyponatremic rats than in normonatremic rats after 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 (Verbalis, 1998).

Several studies have now demonstrated markedly different rates of reaccumulation of brain solutes after normalization of hypoosmolality in hyponatremic mice and rats (Lien et al., 1991; Verbalis and Gullans, 1993). 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. In contrast, after recovery from hyponatremia organic osmolytes, with the exception of glutamate, return to normal brain contents very slowly over a period of many days, whereas electrolytes reach normal or supranormal contents in the brain within 24 h 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 later in the text), 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 (Heilig et al., 1989; Lien et al., 1990), and it suggests that in this situation these electrolytes are similarly gaining access to the brain rapidly through the CSF and act to stabilize intracellular volume (Cserr et al., 1991). 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.

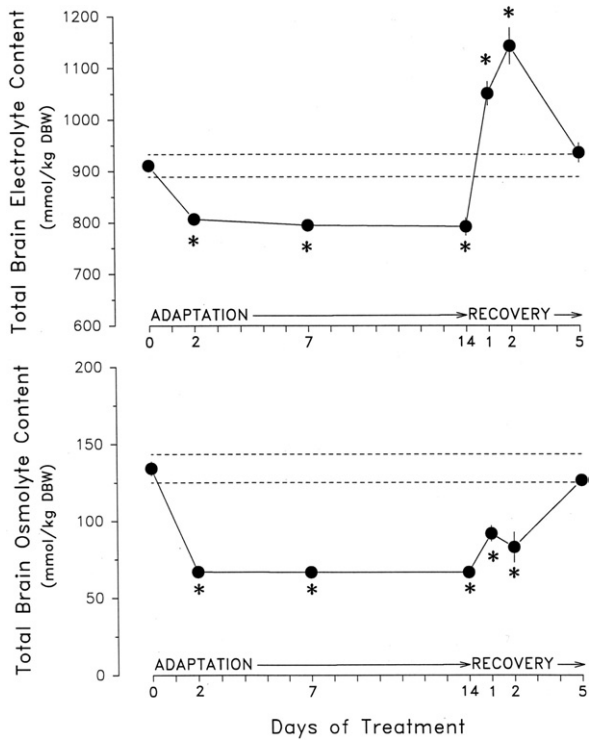

Fig. 3. Time course of changes in brain electrolytes (top panel) and organic osmolytes (bottom panel) during adaptation to chronic hyponatremia and after correction of hyponatremia in rats. Reproduced with permission from (Verbalis, 1998).

Functional and clinical implications of brain volume regulation during hypoosmolality

In addition to physiological implications for brain volume regulation, the large decrease in brain organic osmolyte contents has several potentially important functional and clinical implications.

Neuronal hyperexcitabilty and seizure activity. Hypoosmolality is well known to increase neuronal excitability and seizure activity. Previous electrophysiological studies implicated promotion of epileptiform activity by strengthening both excitatory synaptic activity and cortical field effects (Andrew, 1991). The decrease in brain content of organic osmolytes over relatively short periods (i.e., $<$48 h) now provides a potential mechanism to explain these effects. Because most of these osmolytes losses occur through 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 (Pasantes-Morales et al., 2006; Pasantes-Morales and Tuz, 2006; Verbalis et al., 1995). This hypothesis could also explain, at least in part, the observation that when hypoosmolality is maintained for longer periods, both animals and patients become less symptomatic, because increased brain neurotransmitter concentrations in the extracellular fluid 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.

Decreased synaptic transmission and fine motor activity. Although patients with chronic hyponatremia can achieve nearly complete brain volume regulation with loss of most of the neurological symptoms attributable to cerebral edema, it is erroneous to characterize the brains of such patients as “normal.” Because the solute losses that allow brain volume regulation are sustained over long periods, this represents a state of “allostasis” (the ability of a system to dynamically adapt to varying states to accommodate changing demands) rather than homeostasis. In particular, the loss of up to 30% of brain glutamate (Verbalis and Gullans, 1991) suggests the possibility of decreased synaptic release of excitatory neurotransmitters. Recent studies have shown gait instability and an increased incidence of falls in hyponatremic patients who were felt to be clinically “asymptomatic” by standard neurological testing (Renneboog et al., 2006). An impaired ability to make rapid corrections to postural stimuli could be related to decreased synaptic release of

glutamate, or other neurotransmitters, at the terminals of motor neurons. The practical consequence of increased falls in hyponatremic patients would be an increased rate of bone fractures, particularly in elderly patients. Two studies have now documented a higher incidence of hyponatremia in patients with fractures related to falls (Gankam et al., 2008; Sandhu et al., 2009). Finally, more recent studies have demonstrated resorptive bone losses as a result of hyponatremia itself (Verbalis et al., in press), potentially further increasing the risk of fractures in hyponatremic patients.

Brain demyelination after rapid correction of hyponatremia. Knowledge of this greater inefficiency of brain solute reaccumulation and volume regulation after correction of chronic hyponatremia is very relevant to understanding the pathologic 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 remains unproven, this pathologic disorder likely results from the brain dehydration that has been demonstrated to occur after 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 after subsequent increases in osmolality. This, in turn, can lead to pathologic demyelination. Further support for dehydration-induced demyelination has come from reports that acute hyperosmolality can also cause demyelination in experimental animals (Soupart et al., 1996), although larger increases in plasma osmolality are required than in hyponatremic rats. Although the exact mechanisms responsible for production of brain demyelination after 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. Magnetic resonance studies in animal have shown that chronic hypoosmolality predisposes rats to opening of the blood-brain barrier after rapid correction of hyponatremia, and that the disruption of the blood-brain barrier is highly correlated with subsequent demyelination (Adler et al., 2000). A potential mechanism by which blood-brain barrier disruption might lead to subsequent myelinolysis is through an influx of complement, which is toxic to the oligodendrocytes that manufacture and maintain myelin sheaths of neurons, into the brain (Baker et al., 2000). Further evidence for this mechanism is provided by recent studies showing that treatment with high doses of glucocorticoids, which stabilize the blood-brain barrier, can significantly decrease the demyelination seen after rapid correction of hyponatremia in rats (Sugimura et al., 2005).

HYPEROSMOLALITY

Hyperosmolality and hypernatremia usually occur 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. 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 (Arieff, 1984). The neurological symptoms of hypernatremic states are a result of the cellular dehydration produced by osmotic shifts of water from the intracellular fluid space into the more hypertonic extracellular fluid space. The symptomatology is related both to the severity of the hyperosmolality and also to the rate at which it develops (Palevsky, 1998). The symptoms of hyperosmolar states are a consequence of neurologic dysfunction resulting from cellular dehydration. These symptoms include irritability, restlessness, stupor, muscular twitching, hyperreflexia, spasticity, and in severe cases, seizures, coma, and ultimately death (Arieff, 1984).

Brain adaptation to hyperosmolality

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 additional solutes to restore the brain volume to its normal level, termed a *volume regulatory increase (VRI)* in solutes. This adaptation process involves rapid accumulation of inorganic ions and slower accumulation of organic osmolytes, traditionally termed "idiogenic osmoles." Marked differences in symptoms, and hence in recovery from hyperosmolality, exist because of the complex, time-dependent nature of this brain adaptation. Optimal treatment of hyperosmolar patients is facilitated by knowledge of the basic mechanisms underlying the process of adaptation to the hyperosmotic state. As for hyponatremia, neurological symptoms and mortality are generally higher in patients with acute rather than chronic hypernatremia, and therefore, it is useful to consider brain adaptation to these different pathologic states separately.

Adaptation to acute hyperosmolality. Acute hypernatremia, generally defined as the development of serum [Na^+]$>$145 mmol/liter in 24–48 h, 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 rarity of acute hyperosmolality, it is important to understand the pathophysiology underlying the neurological symptoms because of the marked morbidity and mortality, which can be as high as 75% in adults and 45% in children (Arieff, 1984; Palevsky, 1998).

Acute hyperosmolality is typically induced in animals by i.p. injections of hypertonic NaCl, 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 of rats, 3 h of hypernatremia ($[Na^+]>200$ mmol/liter) decreased brain water by 14% and promoted increases in contents of Na^+ and Cl^- of 34% and 60%, respectively; K^+ content was unaltered (Holliday et al., 1968). 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 15–30 min after the NaCl injection. However, after 30 and 120 min, the brain water loss was only 35% of that predicted, which indicates that significant volume regulation had already occurred. 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 CSF, whereas the slower rise in tissue K^+ content was related to an influx from plasma across the blood-brain barrier (Cserr, 1988; Cserr et al., 1987).

A central problem in studies of brain volume regulation has been an inability to distinguish the changes that occur in the intracellular and extracellular spaces. A seminal study by Cserr and coworkers used ion-selective electrodes to resolve this issue (Cserr et al., 1991). Rats, given a single i.p. injection of NaCl, experienced a 7% loss of brain water at 30–90 min, but this loss was related entirely to a decrease in extracellular water content; intracellular water content remained normal. Estimates of intracellular and extracellular ion contents indicated that extracellular Na^+, Cl^-, and 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 appear to contribute significantly to brain volume regulation (Chan and Fishman, 1979; Lien et al., 1990). Thus, acute hypernatremia is characterized by rapid loss of total brain water, and maintenance of intracellular volume by rapid accumulation of electrolytes from extracellular fluid, CSF, and plasma.

Adaptation to chronic hypoosmolality. In most hypernatremic patients, hyperosmolality develops gradually over a period of several days, regardless of the etiology. Although morbidity and mortality rates are reported to be relatively high in both adults and children, interpretation of these findings is difficult because death is very often a result of the underlying disease that caused the fluid imbalance. 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. This ability has been attributed to slow accumulation of organic solutes, and recent studies have provided greater understanding of this adaptation process.

In animals, when hypernatremia persists beyond several days, total brain tissue water content slowly returns to normal levels. This restoration of total brain water does not result from continued accumulation of electrolytes but rather from accumulation of specific organic osmolytes. The organic osmolyte accumulation generally accounts for 30%–50% of the solute accumulation in hypernatremic animals. Organic osmolytes involved in volume regulation are amino acids, methylamines, and polyols. The major organic osmolytes in the brain are glutamine, glutamate, taurine, and myo-inositol. Organic osmolytes of lesser significance include several other amino acids, two methylamines glycerophosphorylcholine (GPC) and betaine, phosphocreatine/creatine, and the neurotransmitter GABA. 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 to adaptation to hypernatremia (Fig. 4).

Organic osmolytes accumulate in the brain slowly after 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 9–24 h and reach a steady-state level at 2–7 days. In vitro studies of cultured brain cells corroborated this delayed and slow rate of organic osmolytes accumulation. The mechanisms of brain organic osmolytes accumulation remain poorly understood, but likely represent a combination of uptake (e.g., myo-inositol and taurine) and synthetic mechanisms (e.g., glutamate), as described in the renal medulla. Circulating blood levels of many amino acids are increased during hypernatremia, and these may serve as a precursor pool for brain osmolytes. An issue that remains to be resolved is the intracellular/extracellular distribution of organic and inorganic osmolytes in chronic hypernatremia. Studies of other systems would suggest that the organic osmolytes are preferentially accumulated intracellularly and thus replace the inorganic solutes, which are responsible for the acute phase of brain cell volume regulation. Recent findings that the tonicity-responsive-binding protein (TonEBP) is substantially up-regulated in neurons in response to systemic hypertonicity suggest that neurons may accumulate compatible osmolytes through TonEBP-mediated activation of osmoprotective genes as the sig-

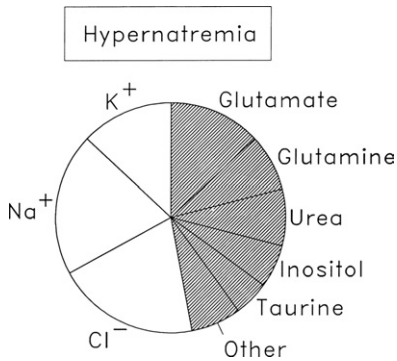

Fig. 4. Relative increases in individual brain electrolytes and organic osmolytes during adaptation to chronic hypernatremia in rats. The category "other" represents GPC, urea, and several other amino acids. Reproduced with permission from (Gullans and Verbalis, 1993).

naling mechanism that allows adaptation to systemic hypertonicity (Loyher et al., 2004).

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 as a result of 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 may occur. When acute hypernatremic 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 hypernatremic episode. In contrast, during recovery from chronic hypernatremia, restoration of brain organic solute contents to normal levels occurs slowly over 24–48 h. Studies in rats found that brain betaine content fell to normal levels within 24 h, whereas glutamine, glutamate, taurine, phosphocreatine, and GPC took 2 days to achieve normal levels, and myo-inositol remained significantly elevated even after 2 days. The mechanisms responsible for organic and inorganic solute loss during recovery from hypernatremia are not known.

Functional and clinical implications of brain volume regulation during hyperosmolality

In addition to physiological implications for brain volume regulation, the accumulation of brain organic osmolyte contents with hyperosmolality also has potentially important functional and clinical implications.

The slow dissipation of accumulated organic solutes is the basis for clinical recommendations that chronic hypernatremia be corrected relatively slowly over 48 h. In support of a slow correction, studies of children reported a high incidence of seizures after rapid correction of severe hypernatremia, presumably caused by brain edema, as seen in animals. Although well-controlled studies of optimal correction rates in adults do not exist, it seems prudent to continue to recommend the more cautious approach of prompt but gradual correction of chronic hypernatremia on the basis of what is known about the rates of organic solute losses from brain tissue in animals.

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