Brain Myelinolysis Following Hypernatremia in Rats

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Abstract. Brain myelinolysis could develop after excessive correction (ΔSNa > 20–25 mEq/l/24 hour (h)) of chronic hypernatremia; however, this neurological event is not recognized as a complication of hypernatremia when arising from a normonatremic baseline. Previous animal studies were unable to reproduce these brain lesions in hypernatremia after acute increase of serum sodium to moderately hypernatremic levels. We hypothesized that to produce brain dehydration and myelinolysis from normonatremic baseline requires a more important osmotic gradient than when starting from hypernatremic state. Rapid and sustained hypernatremia (at least >6 to 12 h) was induced in male rats by i.p. administration of NaCl 2 M (3 injections at 6 h intervals). The NaCl doses were determined to define two groups of hypernatremic rats (moderate and severe hypernatremia) for further analysis of the neurological outcome. In group 1 (moderate hypernatremia, n = 26) 8 rats died early (<12 h) after the beginning of the NaCl administration without specific neurologic manifestations. All the surviving rats fared well and were asymptomatic at time of death (day 8). They were submitted for at least 6 to 12 h to a serum sodium gradient of 28 ± 6 mEq/l. Brain analysis was normal in all of them without brain demyelinating lesions. In group 2 (n = 51), 24 rats also died rapidly (<12 h). The surviving rats developed severe neurologic symptoms as typically encountered in hypernatremic rats with myelinolysis. The majority of them died before day 8. The hypernatremic gradient in this group was significantly higher than rats in group 1 that completely recovered (mean ΔSNa: 33 ± 3 mEq/l). Brain analysis demonstrated severe demyelinating lesions similar to the histologic changes observed in hypernatremia-related myelinolysis. We demonstrated for the first time that high and sustained levels of hypernatremia could induce brain myelinolysis and that the osmotic gradient necessary to produce brain lesions is higher for normonatremic than for hypernatremic rats.

Key Words: Central pontine myelinolysis; Hypernatremia; Hyponatremia; Osmotic demyelination syndrome.

INTRODUCTION

The development of brain demyelinating lesions after excessive correction of chronic hypernatremia is now a well-recognized problem (1–3). This complication has been attributed to excessive brain dehydration as a consequence of the osmotic stress produced by the serum sodium rise (3–7). Surprisingly, however, when hypernatremia develops without preceding hypotension, this osmotic stress does not produce brain demyelination. Brain dehydration occurs to a greater degree in hyponatremic than in normonatremic rats after similar increase in osmolality (4, 5). It has been suggested that this higher susceptibility of the hypothalamic brain to an osmotic stress reflects a lower osmotic buffering capacity due to brain solute losses during adaptation to hypernatremia (4–8). Different mechanisms have been proposed to explain the development of brain demyelination after an osmotic insult. Excessive dehydration can lead to shrinkage of brain cells, and mechanical disruption of axons away from their myelin sheaths has been recently described (9, 10) in hypernatremic rats occurring 30 minutes (min) after an osmotic stress. Other pathogenic hypotheses also implicate the osmotic opening of the blood-brain barrier and the potential release of myelinotoxics factors (11, 12). It is also possible that the overshooting of brain electrolyte content observed after the excessive correction of hypernatremia (7, 8) could be detrimental for brain cells, thereby participating also in the pathogenesis of myelinolysis.

Recently, animal (rat) studies have contributed to define the risk factors for brain demyelination in hypernatremia. It is now clear that brain lesions appear in rats when the magnitude of correction of the serum sodium (ΔSNa) exceeds the threshold of 20 mEq/l/24 hours (h) when correction is achieved over 48 h (13) or 25 mEq/l/24 h for a correction over 24 h (14). We also know that the rate of correction is not a critical determinant of the neurological outcome if the daily rise in serum sodium remains below the threshold of 20 mEq/l/24 h (15). Above this limit, the rate of correction probably represents an additional risk factor (16).

In man, myelinolysis has been occasionally reported in normonatremic patients exposed to hyperosmolar states, including hypernatremia. In the great majority of the cases, however, these patients had other predisposing conditions known to be associated with the development of central pontine myelinolysis (17–22). On the other hand, previous animal studies were unable to reproduce brain myelinolysis in hypernatremia from a normonat-

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brain demyelination and hypernatremia

tremic baseline after an acute increase of the serum sodium to moderately hypernatremic levels (1, 14, 23–25). This apparent “resistance” of the normonatremic brain to the osmotic injuries might in fact be related to insufficient increase in serum sodium in previous experiments and to short-lived hypernatremia. Indeed, on a theoretical basis, one can hypothesize that to produce brain dehydration in normonatremia requires a more significant osmotic stress than in a hyponatremic state because of the higher osmotic content of normonatremic cells. We recently demonstrated that the osmotic stress in hyponatremic rats must be maintained for a sufficient period of time to induce brain lesions (26). We tested this hypothesis in rats by studying the effects of an acute (<24 h), large and sustained (>6 to 12 h) hypernatremia on the neuropathological outcome.

MATERIALS AND METHODS

Animals

All the experiments were performed using male Wistar rats weighing 280–450 grams (g). All the rats were housed individually in their cages and were allowed 10 days to adapt before starting the study, with free access to tap water and pelleted rat chow. Room temperature was controlled (20°C) with lights on from 7:00 A.M. to 7:00 P.M.

Induction of Rapid and Sustained Hypernatremia

Two groups of rats were investigated, according to the severity of the hypernatremia. The salt loading was achieved by intraperitoneal injections of hypertonic saline. 2 M NaCl. The doses and timing of NaCl administration were determined by pilot experiments in order to obtain a mean increment in serum sodium (ΔSNa) of approximately 25 mEq/l in one group (group 1: moderate hypernatremia) and 35 mEq/l in the second group (group 2: severe hypernatremia), and to maintain this osmotic stress for at least 6 to 12 h.

The rats received an initial dose of 2 M NaCl (day 1) via intraperitoneal injection at a dose of 2 ml/100 g b.w. in both groups. Additional injections of 2 M NaCl were administered 6 h and 12 h after the first injection at doses of 0.75 ml/100 g b.w. (6th hour) and 0.75 ml/100 g b.w. (12th hour) for group 1 (n = 26), and 1 ml/100 g b.w. (6th hour) and 1 ml/100 g b.w. (12th hour) for group 2 (n = 51), respectively. In group 1, rats with a ΔSNa ≥ 25 mEq/l at the 12th hour were not reinjected at that time. During the experiments, all rats were allowed free access to food, but water was removed during the first 24 h of the protocol.

Blood samples were collected via tail transection for serum sodium determination just before the first NaCl injection and then, after 6 h for 8 rats in both groups, at 12 h and 24 h for all the rats. Animals were kept under light ether–ether anesthesia at time of injections and blood sampling. During the experiments the rats were observed and the neurological abnormalities were noticed. At day 8, rats were decapitated and the brains were dissected and weighed. In group 1, the serum sodium was measured in serum collected from trunk blood. The serum sodium was measured on these samples after centrifugation (3,000 × g for 10 min) via ion-specific electrodes (Microlyte, Kone, Espoo, Finland). Body weights were measured initially, before starting the experiment, after the first 24 h and at time of the death (day 8). In 8 rats submitted to the same protocol but not included in the 2 groups, oxygen tension (pO2) and carbon dioxide tension (pCO2) were measured by automated analyser (ABL90, Radiometer, Copenhagen, Denmark) immediately after drawing arterialized blood samples collected via tail transection (27). Serum sodium and blood glucose concentrations were measured concomitantly.

Histological Examination of the Brains

At day 8, surviving rats were decapitated. Skulls were opened and the brains were extracted and immediately placed in 10% buffered formaldehyde for 10 days. Brains were sectioned coronally at 6 levels and processed for light microscopy as previously described (13). Briefly, brain sections were stained with haematoxylin–eosin for evaluation of neuronal density and Luxol fast blue for analysis of the myelin integrity. The studies were independently analyzed by two neuropathologists (OR AST) without knowledge of the treatment group of the animals.

Statistical Analysis

All values are expressed as means ± SD. To determine statistical difference of various parameters between different groups, conventional t-tests were used.

RESULTS

As shown in Table 1, the hypernatremic gradient was approximately 10 mEq/l higher in group 2 than in group 1 at the twelfth hour and during the following period. The ΔSNa value obtained at the 6th hour could be considered as the serum sodium gradient already reached at the first hour. Indeed, after intraperitoneal injection of hypertonic saline, the level of serum sodium rises very rapidly (30 min to 1 h) (13, 28). In Figure 1, the individual data in each group represents the higher increment in serum sodium sustained for at least 6 to 12 h and observed during either the first (0 to 12 h) or the second (12 to 24 h) twelve-hour period.

In group 1 (moderate hypernatremia, n = 26), the serum sodium concentration before hypertonic saline injection was 138 ± 1 mEq/l. Eight rats died rapidly before

<table>
<thead>
<tr>
<th>ΔSNa (mEq/l)</th>
<th>6 hours</th>
<th>12 hours</th>
<th>24 hours</th>
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<tr>
<td>Group 1</td>
<td>28 ± 3</td>
<td>25 ± 7</td>
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<tr>
<td>(n = 8)</td>
<td>(n = 18)</td>
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<tr>
<td>Group 2</td>
<td>28 ± 7</td>
<td>35 ± 11*</td>
<td>36 ± 11**</td>
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<tr>
<td>(n = 8)</td>
<td>(n = 26)</td>
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<td>(n = 22)</td>
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Data are mean ± SD. * p < 0.005 and ** p < 0.001 as compared to group 1.
weight and were free of neurological sequelae. Brain analysis in all of them was normal.

In group 2 (severe hypernatremia, n = 51), the initial serum sodium concentration was 139 ± 1 mEq/l. All the rats in this group developed severe clinical symptoms and physical signs related to the hyperosmolar state: muscle irritability, muscular twitching and trembling, tonic spasm, and varying degrees of lethargy and stupor. During the first 12 h of the hypernatremic state, some of these rats (n = 24) became rapidly comatose without more characteristic neurological findings and died. No rats presented symptoms or signs consistent with pulmonary edema. Among the remaining rats (n = 27), another group of rats (n = 11) rapidly developed (<12 h) neurologic symptoms similar to those typically encountered in rats presenting brain demyelination after excessive correction of chronic hyponatremia (13, 16). These clinical manifestations were characterized by the sudden onset of hyperactivity, hyperirritability (the rats jumping around the cages spontaneously or after stimulation and hitting themselves violently against the walls), ataxic gait, spontaneous crying, spasticity of the extremities, and paralysis of the limbs. These early symptomatic rats did not receive the third injection of NaCl at the 12th hour. The gradient of serum sodium increase for these rats observed at that time (12th hour) was ΔSNa: 43 ± 10 mEq/l (mean ΔSNa: 183 ± 10 mEq/l, n = 11). Four of these rats, which were about to die (all of them comatose and unresponsive to tactile stimuli), were sacrificed. Lung appearance and weight were normal in all of them (mean weight of both lungs: 1.79 ± 0.18 g) with no evidence of pulmonary edema. Brain analysis was performed and the histological examination was normal in the 4 rats.

We also measured in 6 additional rats not included in the other groups the hematocrit values and the plasma protein levels in normonatremia and 2 h after an i.p. injection of 2 M NaCl. Hypertonic saline injection was followed by a decrease in both hematocrit (44 ± 0.7 vs 39 ± 1.7%, p < 0.01) and plasma protein concentration (6.3 ± 0.2 vs 5.9 ± 0.2 g/dl, p < 0.01) after 2 h of hypernatremia (SNa: 174 ± 13 mEq/l). This is likely explained by the circulating volume expansion state induced by the NaCl administration.

After 12 h of sustained hypernatremia, the 16 remaining rats had a ΔSNa of 30 ± 8 mEq/l (mean SNa: 169 ± 8 mEq/l) and after 24 h the ΔSNa was 35 ± 9 mEq/l (mean SNa: 175 ± 9 mEq/l). All except 2 of the rats also developed neurological symptoms suggesting brain demyelination (evident for the majority (12/16) after 24 h). At that time, their body weight had decreased by 16%. During the subsequent days, all the rats in group 2 had a catastrophic clinical outcome and only 7 of the 27 rats reached day 8 for brain analysis, the other 20 rats dying during the first 72 h after the onset of severe hypernatremia. They were found dead in their cages and their brains

Fig. 1. Relationship between the extent of serum sodium increase sustained for at least 6 to 12 h and the development of neurological symptoms and brain myelinolysis in hypernatremic rats. In group 1 (moderate hypernatremia, n = 18, mean value of increase in serum sodium, ΔSNa: 28 ± 6 mEq/l, ▲) all the rats remained asymptomatic and the brain analysis was normal. In group 2 (severe hypernatremia, n = 27, mean value of ΔSNa: 39 ± 8 mEq/l) the rats developed neurological symptoms consistent with brain myelinolysis and the majority of them died rapidly (○). In the 7 surviving rats, brain analysis demonstrated typical brain demyelination (●).
were not analyzed in order to avoid problems with autolysis artefacts.

As compared to group 1, the maximum increment in serum sodium sustained during at least 6 to 12 h in group 2 was significantly higher (39 ± 8 mEq/l vs 28 ± 6 mEq/l, p < 0.001) (see Fig. 1); with a mean serum sodium of 179 ± 8 mEq/l vs 166 ± 6 mEq/l (p < 0.01). It must be noted that no major changes in the pO₂ (mean pO₂: 69.4 ± 9 mmHg range 56–86 mmHg; mean pCO₂: 38 ± 7 mmHg) and no hyperglycemia (starting value: 193 ± 21 mg/dl, and 189 ± 12 mg/dl in hyponatremia) developed during the induction of the hyponatremia, as documented in the 8 rats submitted to similar levels of hyponatremia (mean serum sodium: 172 ± 17 mEq/l, n = 8).

Among the 7 surviving rats on day 8, in this group two were free of neurological symptoms at the time of the death. The maximum ΔSNa supported by these rats during a protracted (6–12 h) period of time was 33 ± 3 mEq/l (n = 7, p < 0.05 as compared to group 1 and p < 0.02 as compared to the rats with early death, n = 20, mean ΔSNa: 42 ± 8 mEq/l).

Brain analysis of these 7 rats demonstrated histological changes similar to the neuropathological lesions observed in brain myelinolysis related to excessive correction of hyponatremia with similar topographic distribution (1, 13, 14, 16, 24). Symmetrical demyelinative lesions involved the thalamus and basal ganglia (5/7), tegmentum (4/7), claustro-striatum-external capsule (1/7), cerebellum (1/7), cerebral cortex (2/7), and the hippocampus (4/7). Histopathological lesions consisted in area of demyelination with spongy changes, gliovascular proliferation, foamy macrophages infiltration, and neuronal loss (Figs. 2A, B, 3). No petechial intracerebral hemorrhages or subdural haematoma were noticed in these rats; however, they survived up to day 8 and were submitted to a lower extent of osmotic gradient.

**DISCUSSION**

This work demonstrates for the first time that brain myelinolysis could be produced by acutely (<24 h) increasing the serum sodium to large and sustained (at least >6 to 12 h) hypernatremic levels in rats without preceding hyponatremia. During the development of the hypernatremia, the severity of the symptoms are generally grossly correlated with the rate at which serum sodium increases and with the extent of the hypernatremic gradient (29, 30). In animals (rabbits) with acute (<24 h) hypernatremia (time of maximal hypernatremia 1 to 9 h), mortality rate of 76% was observed for a mean serum sodium osmolality of 369 mosm/kg H₂O (30, 31). Major symptoms (seizures, coma) and death generally appear when effective plasma osmolality exceeds 400 mosm/kg H₂O (30, 31). Acute hypernatremia could produce anatomical brain damage with petechial hemorrhages, subdural hematoma, and these haemorrhagic complications are generally correlated with the time taken to accomplish maximal (high levels) hypertoncity (30). A significant proportion of our rats, mainly in group 2 in which we observed the higher osmotic gradients, died early during the induction of hypernatremia. These rats became rapidly comatose without other more specific neurologic symptoms and this fatal outcome was probably the consequence of the complications directly associated with the hypertoncity (29–31).

On the other hand, this model of rapid (acute) development of large and sustained hypernatremia has provoked in other rats the early appearance of severe neurologic symptoms as typically encountered in hyponatremic rats with brain myelinolysis following excessive correction of the serum sodium: hyperexcitability (the rats jumping around the cages spontaneously or after stimulation and hitting themselves violently against the wall), paralysis of the limbs, ataxic gait. These symptoms have never been previously described in severely hypernatremic animals (29, 30). We did recently show in hyponatremic rats that rapid and large correction of serum sodium could be similarly followed by early (4 to 12 h after NaCl injection) neurologic manifestations (26), these symptoms attesting to the subsequent development of brain myelinolysis (16, 26, 32) (almost all these rats died shortly after the onset of the neurologic findings if their serum sodium was not rapidly lowered) (26, 33). Thus we observed the same phenomenon, at a higher level of ΔSNa, in a subset of hypernatremic rats (group 2) with a high mortality rate (83%) in those exhibiting early (<24 h) myelinolysis-related symptoms. The surviving rats (5/7 presented symptoms) had a lower magnitude of hypernatremia (ΔSNa: 33 mEq/l vs 42 mEq/l) and presented histological changes similar to the brain injuries usually described in hyponatremic rats with brain myelinolysis.

It must be noted that no major hypoxia could be documented in this model despite high hypernatremic levels. In fact, hypoxia is very unlikely to be involved in the pathogenesis of the clinical and neuropathologic findings observed in our experiments. No rats, and particularly none of the 7 rats with documented brain myelinolysis, developed symptoms consistent with pulmonary edema or respiratory failure. Previous animal studies with severe acute hypernatremia also failed to demonstrate clinical manifestations or histological changes suggesting potential association with hypoxic injuries (1, 14, 23, 25, 29, 30). Moreover, normal rats submitted to severe (pO₂ 35 mmHg) and sustained (3 h) hypoxia, completely recovered without brain lesions (34). Finally, the clinical symptoms and the neuropathological changes observed in both hyponatremic and hypernatremic rats appear to be characteristic of the osmotic demyelination syndrome (26). Nevertheless, as we didn't measure the cerebral blood flow during hypernatremia in our model, we cannot
Fig. 2. A. Coronal section through the thalamic region with bilateral symmetrical well-circumscribed zones of palor indicating demyelinating and reactive lesions (Luxol fast blue). B. Normal control (Luxol fast blue).
count out the possibility that some degree of cerebral ischemia also contributes to the pathogenesis of the brain damage.

In the previous experimental models, the role of hyponatremia in the genesis of brain myelinolysis was in fact poorly studied, with few data about the kinetics of serum sodium increase (1, 14, 23–25). The magnitude of the shift in serum sodium was generally less than 25 mEq/l and the osmotic stress was maintained only for several hours (single i.p. injections of 1 M NaCl). We showed that the rats developing neurological symptoms of brain myelinolysis and/or brain lesions have been submitted to a magnitude of serum sodium increase largely above (39 mEq/l) the threshold for myelinolysis defined in hyponatremic rats (20–25 mEq/l/24 h). The threshold of absolute change in serum sodium for inducing brain lesions in normonatremic rats, although not precisely defined, seems to be approximately 30–35 mEq/l, which represents roughly a 50% increase in the critical ΔSNa as compared to hyponatremic rats (13, 14, 16). In terms of osmotic stress on the cell, our findings suggest that the osmotic gradient supported by the brain in hyponatremia and in normonatremia are equivalent if we take the change as a proportion of the osmotic increase: indeed, an increase in serum sodium from 100 to 125 mEq/l (ΔSNa: 25 mEq/l) and an increase from 135 to 172.5 mEq/l (ΔSNa: 37.5 mEq/l) both represent an equivalent proportional increase in osmolality (25%). Thus, this suggests that the critical factor for shrinkage of brain cells and secondary myelinolysis is determined by a proportional rather than by an absolute value of the osmotic increase. This parameter is probably a constant value for the brain, whatever the initial serum sodium level.

As observed in hyponatremic rats in previous studies (13, 16), there is some overlap between the two groups, showing individual variability in the production of demyelinative lesions either in hyponatremic rats or now in normonatremic rats as well. This higher susceptibility of the hyponatremic brain cell to a hyperosmotic stress, which leads to a larger cell volume change (shrinkage) compared to normonatremic baseline, could theoretically be explained by the lower osmotically active brain solute content of the hyponatremic cell.

In both normonatremic and hyponatremic rats, the response to acute increase in osmolality similarly consists first in rapid electrolyte shifts (Na⁺, Cl⁻ and K⁺) into the
brain, followed by delayed reaccumulation of organic osmoles (7, 8, 28, 29, 35–40). Thus, in both situations (normo- and hyponatremia), this volume-regulating process could be theoretically overwhelmed. Obviously, during acute hypernatremia (ΔSNa: 35–55 mEq/l) the brain loses water and shrinks (36, 38), although less than predicted for ideal osmotic behavior (28, 37). In a recent work, however, Cser et al have demonstrated in hypernatremic rats that brain dehydration is accomplished entirely through removal of water from the extracellular compartment while intracellular volume is maintained (37). In fact, one of the currently proposed hypotheses is that myelinolysis follows excessive brain dehydration, with shrinkage of axons away from their myelin sheaths as initial insult (3, 9, 10). This mechanism probably implies intracellular dehydration as a consequence of the osmotic stress. However, in the model of Cser et al, the osmolality was increased up to roughly 360 mosm/kg H₂O during 90 min which is probably insufficient (borderline), according to our results (mean ΔSNa: 30–35 mEq/l during at least 6 to 12 h) (26), to dehydrate the intracellular compartment. In this sense, the levels of hyperosmolality reached in the experiments of Cser et al are also below the threshold for osmotic opening of the blood-brain barrier (385 mosm/kg H₂O) (40), a mechanism which has also been implicated in the pathogenesis of brain demyelination (11, 12).

In clinical settings, brain myelinolysis (central pontine and extrapontine myelinolysis) has been only rarely reported in association with hypernatremia without preceding hyponatremia (17, 22). According to our results, this is not surprising, knowing that theogenesis of brain myelinolysis necessitates the rapid development of high levels of sustained hypernatremia. Acute (<24 h) hypernatremia develops in fact in rare and extreme circumstances, mainly in the pediatric practice as a result of accidental salt poisoning or gastroenteritis, and very infrequently in adults (generally iatrogenic or after untreated diabetes insipidus in patients unable to drink) (29, 41). There are, however, several other published cases of brain myelinolysis in patients presenting a hyperosmolality of other origin (mainly hyperglycemia, frequently concomitant to the hypernatremia), and/or almost always associated with the presence in these patients of other predisposing factors suspected to play a role in the pathogenesis of “central pontine myelinolysis” (alcoholism, malnutrition, liver disease, several debilitating illnesses) (17, 22, 42–44). These patients with underlying metabolic disorders appear to be particularly susceptible to osmotic injury and these predisposing conditions probably decrease the osmotic threshold for brain demyelination in the metabolically compromised host, as illustrated by various published cases (19–21).

In this experimental model of hypernatremic brain myelinolysis, we showed for the first time that brain de-

myelinolysis could occur after rapid and sustained (6–12 h) hypernatremia and this, after a higher osmotic threshold (ΔSNa ≈ 35 mEq/l) than for hypernatremic rats (ΔSNa ≈ 25 mEq/l/24 h). The fact that such a change produces a myelinopathy supports the view that it is the osmotic stress that is responsible, as also suggested by the observation of brain myelinolysis arising in hypernatremic rats after a mannitol-induced osmotic stress without concomitant correction of the serum sodium (45).

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