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Neurological and neuropathological sequelae of correction of chronic hyponatremia

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Neurological and neuropathological sequelae of correction of chronic hyponatremia. The effect of correction of chronic hyponatremia at different rates was studied in 91 rats maintained at a plasma [Na^+] of 112 ± 1 mmol/liter for 19 ± 1 days. Hyponatremia was corrected into normal ranges (140 to 145 mmol/liter) using three different methods. Rats corrected by water restriction achieved normal plasma [Na^+] by 2.1 ± 0.2 day and had a maximal (4 hr) correction rate of 1.0 ± 0.1 mmol/liter · hr; rats corrected by water diuresis achieved normal plasma [Na^+] by 1.6 ± 0.1 day and had a maximal correction rate of 2.8 ± 0.2 mmol/liter · hr; rats corrected by hypertonic saline infusion achieved normal plasma [Na^+] by 5.4 ± 0.3 hr and had a maximal correction rate of 5.7 ± 0.4 mmol/liter · hr. A fourth control group was not corrected. No demyelinative lesions were found in the brains from the uncorrected rats, whereas the occurrence of such lesions in the brains of the corrected rats was highly correlated with the maximal rate of increase in plasma [Na^+] ($r = 0.68$, $P < 0.001$), and to a lesser degree with the magnitude of the increase in plasma [Na^+] over the first 24 hours of correction ($r = 0.41$, $P < 0.001$). Brain myelinolysis was first observed in animals whose maximal (4 hr) rate of correction exceeded 1.75 mmol/liter · hr, and the incidence of demyelination increased progressively in rats with more rapid rates of correction. Similarly, myelinolysis was first observed in rats whose magnitude of correction at 24 hours exceeded 16 mmol/liter and also increased in rats with larger 24 hour magnitudes of correction. Analysis of the incidence of myelinolysis using both of these correction parameters together indicated that demyelination did not occur in rats whose maximal rate of increase in plasma [Na^+] did not exceed 4 mmol/liter · hr *and* whose absolute increase in plasma [Na^+] did not exceed 25 mmol/liter in the first 24 hours; for all rats who exceeded *either one* of these two limits the incidence of demyelinative lesions was 64%. Our results suggest that the maximal rate as well as the magnitude of correction of plasma [Na^+] represent significant risk factors for the development of brain myelinolysis after correction of experimental hypoosmolality in chronically hyponatremic rats.

Both hyponatremia and rapid correction of hyponatremia can cause neurological dysfunction and neuropathological damage in humans [1–4]. However controversy still exists with regard to the relative degree to which each of these factors contributes to the morbidity and mortality associated with hyponatremia in human patients [4–8]. In particular, there is disagreement about the relation between correction of existing hyponatremia and the production of pontine and extrapontine brain myelinolysis. Although this disorder has been reported relatively infrequently, its neurological sequelae can be devastating to affected patients [9, 10]. Recent clinical studies have provided substantial evidence supporting both the rate and the magnitude of correction of hyponatremia as important determinants of clinical outcomes following correction of hyponatremia [3–5, 8], but acceptable limits for both parameters remain uncertain at the present time [7].

The rarity of this disorder in combination with the potential gravity of its consequences for individual patients makes further study via controlled clinical trials problematical. For this reason, many animal studies have attempted to address this issue. These studies have uniformly documented that demyelinative lesions can be produced by correction of experimentally-induced hyponatremia in multiple species [11–16]. However, the ease with which demyelination has been produced following correction of hyponatremia in animals stands in marked contrast to the relative rarity of reports of this disorder in humans. This discrepancy between the clinical and experimental experiences raises questions as to the relevance of animal studies of hyponatremia to human disease.

Review of the reported animal experiments to date reveals three common characteristics that to varying degrees potentially interfere with interpretation of their results: 1) most previous studies employed relatively short periods of acute sustained hyponatremia, generally from one to four days; 2) many previous studies experienced high mortality (as much as 30 to 50% in some studies) as a result of the acutely-induced hyponatremia even before correction, thus complicating an assessment of whether the correction or the preexisting hyponatremia itself contributed more to the morbidity and mortality following the correction; 3) no previous studies carefully monitored the progress of the induced corrections with sufficient frequency to allow accurate assessment of maximal rates of increase of plasma Na^+ concentration ([Na^+]), and many utilized methods that likely produced rates of correction of hyponatremia in excess of those generally encountered in clinical experience.

The experiments reported here represent an attempt to study the relation between the rate of correction of plasma [Na^+] and

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the development of brain myelinolysis using a newly described model of chronic hyponatremia in rats associated with negligible morbidity and mortality [17]. By inducing corrections of hyponatremia via different methods while closely monitoring acute and chronic changes in plasma [Na^+], we were able to more closely analyze in individual rats the relation between the rate of correction of plasma [Na^+], as well as several other parameters related to correction of hyponatremia, and the subsequent development of demyelinative brain lesions resulting from myelinolysis.

Methods

Animals and maintenance

Male albino rats of the Sprague-Dawley strain (Zivic-Miller, Allison Park, Pennsylvania, USA) weighing 275 to 325 g were used for all studies. They were housed individually in stainless steel wire mesh cages in a temperature-controlled room (22°C) with lights on from 7 a.m. to 7 p.m. All rats were given pelleted rat chow (Wayne Labs, Chicago, Illinois, USA) and tap water ad libitum until initiation of the protocol used to induce and maintain hyponatremia.

Induction of hyponatremia

Hyponatremia was induced and maintained using methods described recently [17]. Briefly, rats were first acclimated to a rodent liquid diet formula supplying 1.0 kcal/ml (AIN-76; Bioserv, Frenchtown, New Jersey, USA). After two to three days, osmotic minipumps (Alzet model 2002; Alza, Palo Alto, California, USA) containing 1-deamino-[8-D-arginine]-vasopressin (DDAVP; Rorer Pharmaceuticals, Fort Washington, Pennsylvania, USA) at a concentration of 10 μg/ml were implanted subcutaneously under methoxyflurane anesthesia. This resulted in a continuous DDAVP infusion rate of 5 ng/hr. On the day of minipump insertion the rats were given 70 ml of AIN-76 at the regular dilution, then 40 ml of a more concentrated AIN-76 mixture supplying 1.9 kcal/ml each day thereafter [17]. Tap water was withheld during the DDAVP infusions. The osmotic minipumps were replaced at 13 day intervals to maintain a continuous DDAVP infusion. All animals were kept hyponatremic for two to three weeks (mean duration of hyponatremia = 19.4 ± 0.3 days) prior to correction of the chronic hyponatremia as described below.

Correction of hyponatremia.

Two days prior to correction of the hyponatremia all rats had indwelling jugular venous catheters inserted using methods previously described [18]. This enabled infusion of hypertonic saline at a controlled rate, as well as frequent blood sampling for measurement of plasma [Na^+]. Blood samples consisted of 0.25 ml of whole blood which were immediately centrifuged (3,000 × g for 10 min at 4°C). Plasma [Na^+] was measured after centrifugation via ion-specific electrodes (Beckman Electrolyte 2 Analyzer, Brea, California, USA). The red blood cells were suspended in an equal volume of 140 mM NaCl and reinfused into the same animals. Four separate groups of rats were studied:

Group I—no correction (NC). Rats (N = 20) were not corrected, but rather continued on liquid formula and DDAVP infusions to maintain the hyponatremia until euthanasia. Plasma [Na^+] was measured daily for six to seven days at which time the rats were euthanized for histological brain analysis.

Group II—water restriction (WR). Rats (N = 23) continued on DDAVP infusions, but the liquid formula was replaced with pelleted chow and tap water ad libitum. Under these conditions rats self-restrict ingested water to the amount required to replace daily losses [19]. Plasma [Na^+] was measured every four hours for the first eight hours after the diet change, and then daily thereafter for six to seven days.

Group III—water diuresis (WD). The osmotic minipumps were removed from rats (N = 26) under light inhalational anesthesia with methoxyflurane, thereby stopping the DDAVP-induced antidiuresis. Animals were switched from the liquid diet to pelleted chow and tap water ad libitum. Plasma [Na^+] was measured every two hours for the first eight hours after the osmotic minipump removal, and then daily thereafter for six to seven days.

Group IV—hypertonic saline (HS). Rats (N = 22) received a continuous intravenous infusion of 2.0 M NaCl at a rate of 1.0 ml/hr via syringe pumps (model A-99; Razel, Stamford, Connecticut, USA). Plasma [Na^+] was measured every two hours during the infusion, and the infusion was stopped once plasma [Na^+] was ≥140 mmol/liter to avoid overcorrection of the hyponatremia. The DDAVP infusions continued as before, but the rats were switched to pelleted chow and tap water ad libitum at the end of the hypertonic saline infusion to prevent recurrence of hyponatremia after the correction. Plasma [Na^+] measurements continued every two hours until eight hours after the start of the infusion, and then daily thereafter for six to seven days.

Rats from all groups were maintained for six to seven days following the correction of hyponatremia (including the uncorrected NC group) before euthanasia for neuropathological analysis. This interval following correction was selected to provide sufficient time for demyelinative lesions to be detected histologically [11]. During this period the rats were closely observed, handled, weighed, and had water intakes measured daily. However, rats were euthanized prematurely before six to seven days if they appeared sufficiently impaired to make survival for the full length of time unlikely. Criteria used for premature euthanasia included loss of more than 25% of body weight, seizure activity, paralysis of the hind limbs with impairment of ambulation, or marked lethargy to the point of unresponsiveness to tactile stimuli. Lesser degrees of neurological dysfunction such as hyperirritability, inadequate grooming (as evidenced by ruffled dirty fur), and decreased spontaneous motor activity were recorded, but were not considered indications for euthanasia.

Neuropathological analysis

Rats were euthanized by decapitation after which the brains were rapidly dissected and immersion fixed in 10% buffered formaldehyde for at least 48 hours. Only rats surviving to the point of euthanasia were included in this analysis; any rats dying prematurely were excluded to avoid autolysis artifacts in the interpretation of the brain histology. The brains were then embedded in paraffin and sectioned transversely at three levels through the brainstem and coronally at three levels through the forebrain. Representative sections (20 to 25 μm) were stained with hematoxylin-eosin for evaluation of neuronal density and

Table 1. Summary of plasma [Na^+] changes, rates of correction, and neuropathology for each treatment group

	Plasma [Na^+] *mmol/liter*			Correction rate *mmol/liter · hr*			Neuropathology			
	Start	Finish	Delta	Max	0–24 hr	0–48 hr	0 (None)	1 (Focal)	2 (Diffuse)	
No correction (NC) $N = 20$	111 ± 2	113 ± 2	2 ± 1	0	0	0	100%	0%	0%	0%
Water restriction (WR) $N = 22$	114 ± 1	142 ± 1	27 ± 1	1.0 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	95%	0%	5%	5%
Water diuresis (WD) $N = 24$	110 ± 2	143 ± 1	33 ± 2[a]	2.8 ± 0.2[b]	1.2 ± 0.1[b]	0.7 ± 0.1[b]	80%	8%	12%	20%
Hypertonic saline (HS) $N = 21$	113 ± 2	142 ± 1	30 ± 2	5.7 ± 0.4[b,c]	1.1 ± 0.1[b]	0.6 ± 0.1	10%	38%	52%	90%

[a] $P < 0.05$, [b] $P < 0.01$ compared to WR
[c] $P < 0.01$ compared to WD

Luxol fast blue for evaluation of myelin integrity. All sections were interpreted by a neuropathologist (AJM) without knowledge of the treatment group of the animals. The presence of brain pathology was graded according to the following scoring system:

0 = no pathological lesions;
1 = focal demyelination (lesions detected in only one brain region);
2 = diffuse demyelination (lesions detected in more than one brain region, including bilateral lesions of the same region).

Statistical analysis

All results are reported as means ± SE. Statistical significance for continuous functions was determined by one-way ANOVA for comparison of multiple groups, with post-hoc comparisons via Bonferroni protected *t*-tests for comparison of paired groups, and for analysis of the frequency of neurological findings in individual animals by chi-square analysis. Correlation coefficients were calculated by the method of Pearson. Two-group discriminant function analysis was performed using the presence (neuropathological score = 1 or 2) or absence (neuropathological score = 0) of demyelinative brain lesions as the dependent variable (GBSTAT software, Dynamic Microsystems, Silver Spring, Maryland, USA).

Results

Results of the four treatment groups are summarized in Table 1. All rats began at levels of plasma [Na^+] that were not statistically different from each other. The Group I (NC) rats remained hyponatremic at equivalently low plasma [Na^+] throughout the six to seven day period before euthanasia (plasma [Na^+] = 113 ± 2 mmol/liter on the day of euthanasia). In contrast, each of the three correction groups eventually achieved plasma [Na^+] that were within normal ranges for rats (defined for our laboratory as 140 to 145 mmol/liter). The total magnitude of the final correction of the plasma [Na^+] was equivalent among the three corrected groups [although the total magnitude of the correction was significantly higher for the Group III (WD) rats than the Group II (WR) rats ($P < 0.05$), neither of these two groups had significantly greater total corrections than the Group IV (HS) rats]. However, the times required to reach these normal ranges differed greatly among the three correction groups. The Group II (WR) rats reached normal levels after 2.1 ± 0.2 day, the Group III (WD) rats after 1.6 ± 0.1 day, and the Group IV (HS) rats after only 5.4 ± 0.3 hr ($P < 0.01$ between each group). These differences in the rates of correction among the treatment groups can be appreciated graphically in Figure 1, which shows the sequentially-measured plasma [Na^+] of each group over the 48 hour period following initiation of correction of the hyponatremia.

Small but different numbers of rats in each group either died spontaneously or had to be euthanized because of weight loss or impaired neurological function prior to six to seven days. In Group I (NC) no animals died spontaneously or had to be prematurely euthanized; in Group II (WR) 1 of 23 (4.3%) died spontaneously; in Group III (WD) 3 of 26 (11.5%) died or were euthanized prematurely; in Group IV (HS) 7 of 22 (31.8%) died or were euthanized prematurely. The spontaneous deaths resulted in the following final numbers of appropriately fixed brains for histological analysis in each group: Group I (NC) $N = 20$, Group II (WR) $N = 22$, Group III (WD) $N = 24$, and Group IV (HS) $N = 21$. All further analysis was restricted to this final study group for whom acceptable neuropathological data was available. Plasma [Na^+] at the time of euthanasia were statistically equivalent in all three of the corrected groups: Group II (WR) plasma [Na^+] = 143 ± 1 mmol/liter; Group III (WD) plasma [Na^+] = 145 ± 1 mmol/liter; Group IV (HS) plasma [Na^+] = 145 ± 2 mmol/liter.

The correction rates averaged over the first 48 hours from the start of the correction were greater in the Group III (WD) than the Group II (WR) rats ($P < 0.01$), but were equivalent between each of these two groups and the Group IV (HS) rats. The correction rates averaged over the first 24 hours from the start of correction were equivalent in the Group III (WD) and Group IV (HS) rats, but for both of these groups were significantly higher than in the Group II (WR) rats ($P < 0.01$). However, when maximal correction rates (defined for this study as the largest increase in plasma [Na^+] averaged over any 4 hr period during the correction) were calculated, they reflected large differences in correction rates across all of the three correction

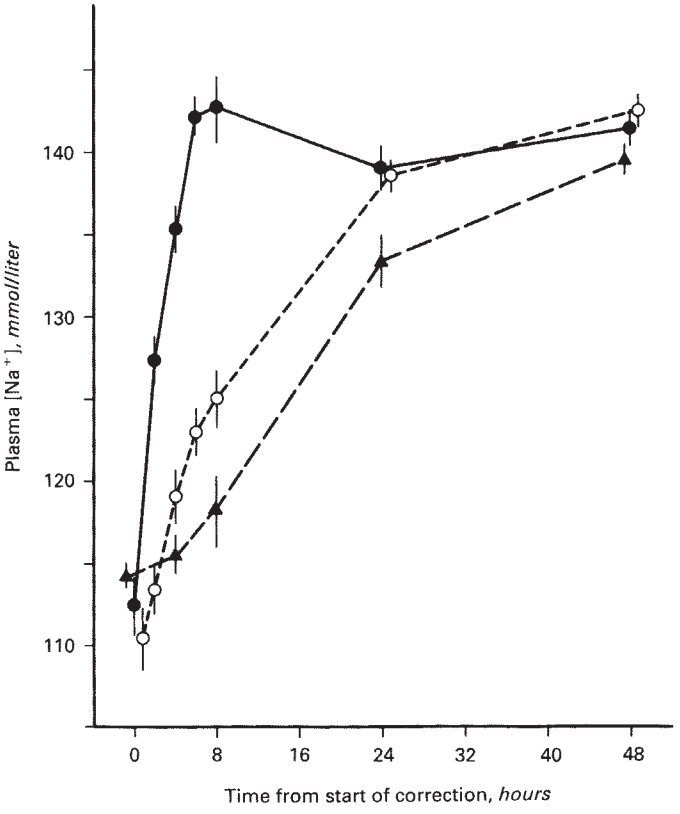

Fig. 1. *Plasma Na^+ concentrations measured sequentially during the first 48 hours of correction of hyponatremia for each of the three treatment groups.* The incremental increases in plasma [Na^+] were measured at the time points indicated for each of the three treatment groups undergoing correction of hyponatremia (–▲–, Group II, water restriction; --○--, Group III, water diuresis; –●–, Group IV, hypertonic saline). Results are shown as means ± SE at each time point.

groups ($P < 0.01$ between each group; Table 1). These marked differences in maximal rates of correction can also be appreciated graphically in Figure 1 by comparing the slopes of the lines depicting the increases in plasma [Na^+] over the first 8 hours of the corrections for each group.

Table 1 also shows a summary of the results of the neuropathological analysis of the rat brains. As with previous studies of demyelination following correction of hyponatremia in rats [11, 13, 15, 16], demyelinative lesions were found predominantly in multiple regions of the forebrain rather than in the brainstem, and in this study particularly in the thalamus and anterior commissure. Demyelinative lesions were found with increasing frequency across the four study groups: Group I (NC) = 0%; Group II (WR) = 5%; Group III (WD) = 20%; and Group IV (HS) = 90%. In addition, the proportion of rats bearing diffuse (neuropathological score = 2) versus focal (neuropathological score = 1) demyelinative lesions similarly increased from Groups II through IV.

Table 2 shows the correlation coefficients calculated between the neuropathological scores and the various parameters that were monitored during the course of the corrections. The presence of demyelinative lesions was weakly, but significantly, inversely correlated with the starting plasma [Na^+] ($r = -0.31$). The total magnitude of the change in plasma [Na^+] was also

Table 2. Correlations between neuropathological scores and correction parameters

	r	*P*
Starting plasma [Na^+]	−0.31	<0.05
Total change in plasma [Na^+]		
0 to 24 hr	0.41	<0.001
0 to 48 hr	0.32	<0.01
0 to end of correction	0.27	<0.05
Correction rate:		
Maximum 4 hr	0.68	<0.001
H_2O intake/24 hr prior to euthanasia	−0.65	<0.001
Weight change following correction	−0.68	<0.001

Table 3. Relation between presence of neurological findings (left) and neuropathological scores in individual rats following correction of hyponatremia ($\chi^2 = 22.82$; $P < 0.0001$)

	Neuropathology score			
	0	1	2	
Absent	41	9	7	57
Present	1	1	8	10
	42	10	15	67

weakly correlated with the neuropathological score ($r = 0.27$), as were the increases in plasma [Na^+] over the first 24 and 48 hours of the correction ($r = 0.41$ and 0.32, respectively). However, the calculated correlation was greatest with the maximal rate of correction over any four hour period as defined above ($r = 0.68$). The increases in plasma [Na^+] over the first 24 and 48 hours were also significantly intercorrelated with the maximal rate of correction ($r = 0.49$ and 0.33, respectively, both P's < 0.01), as expected from the design of the experiments (that is, rats with more rapid rates of correction were generally those who also experienced larger magnitudes of correction).

Analysis of neurological findings following correction of hyponatremia in individual rats showed a significant relation between the presence of any findings (hyperirritability, poor grooming, motor deficits, seizure activity, and unresponsiveness) and their subsequent neuropathological score (Table 3, $P < 0.0001$ by chi-square analysis). These findings were clearly a result of the correction since none of the uncorrected rats manifested any of these findings, as has been reported previously in rats maintained chronically hyponatremic using this protocol [17]. Furthermore, all rats with neurological findings initially appeared quite normal immediately following the correction, and their first manifestation of any sign of abnormality did not occur until 2.2 ± 0.3 days after correction. Although 90% of rats with neurological findings were found to have demyelinative lesions (including all of the rats whose symptomatology was severe enough to warrant premature euthanasia), a lack of findings was not nearly as good a predictor of the absence of such lesions since 28% of asymptomatic rats were also found to have evidence of demyelination (Table 3). High

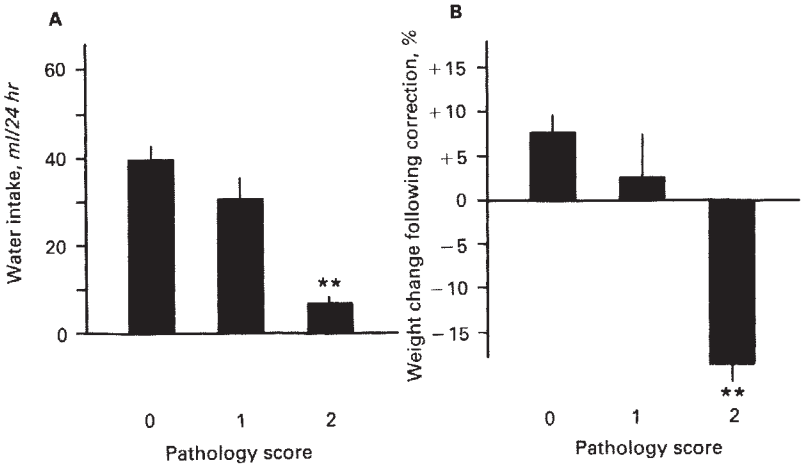

Fig. 2. *Relation between water and food ingestion and the incidence of demyelinative lesions following correction of hyponatremia.* **A.** Mean water intake of rats for the 24 hr period preceding euthanasia. Rats with no demyelinative lesions (pathology score = 0) had an average daily water intake of 40 ± 3 ml, as compared to 31 ± 5 ml in rats with focal lesions (pathology score = 1) and 6 ± 2 ml in rats with diffuse lesions (pathology score = 2). **B.** Mean changes in body weights over the 6 to 7 day period following correction of the hyponatremia expressed as a percent of weight at the start of the correction. Rats with no demyelinative lesions gained 7.7 ± 1.9% over this period, as compared to 2.4 ± 4.8% in rats with focal lesions. Rats with diffuse lesions lost 18.8 ± 2.1% over this period. Results are shown as means ± SE for each group; ** $P < 0.01$ compared to rats with pathology score = 0.

Table 4. Relation between weight change (left) and neuropathological scores in individual rats following correction of hyponatremia ($\chi^2 = 43.90$; $P < 0.0001$)

	Neuropathology score			
	0	1	2	
≤ −15%	1	0	11	12
−1 to −14%	3	2	2	7
≥ 0%	38	8	2	48
	42	10	15	67

inverse correlations were also found between the neuropathological score and ingestive behaviors, specifically water intake over the 24 hour period prior to euthanasia and the total weight change following correction ($r = -0.65$ and -0.68, respectively, Table 2). Figure 2 shows the mean values of these parameters as a function of the neuropathological score, and Table 4 shows that the weight changes also were significantly related to the subsequent neuropathology in individual rats ($P < 0.0001$ by chi-square analysis). Although small degrees of weight loss were not predictive of demyelinative lesions, larger degrees of weight loss (≤15%) were 92% predictive of more diffuse degrees of demyelination. Similar to the absence of neurological findings, weight gain generally predicted the absence of neuropathology, but 21% of rats with weight gain following correction still were found to have demyelinative lesions, although in most cases these were focal (neuropathology score = 1) rather than diffuse in nature (neuropathology score = 2).

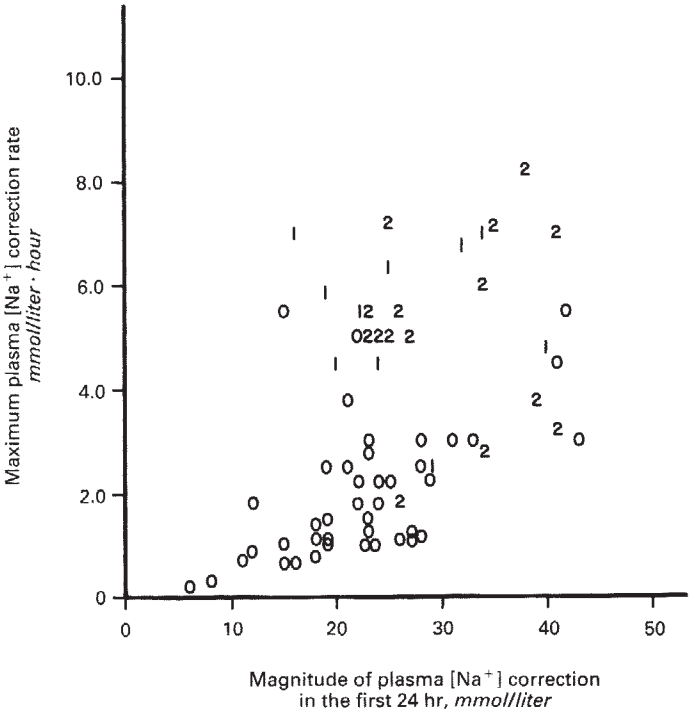

Fig. 3. *Incidence of demyelinative lesions in individual rats as a function of both the maximal rate of correction of hyponatremia and the magnitude of the increase in plasma Na^+ concentration over the first 24 hours of the correction.* Each rat in one of the three correction groups (water restriction, water diuresis, and hypertonic saline) is plotted as a function of the maximal rate of increase in plasma [Na^+] achieved over any 4 hour period during the correction (abscissa) and the total magnitude of the increase in plasma [Na^+] achieved during the first 24 hours of the correction (ordinate). Rats are depicted by their neuropathology score (0 = no demyelinative lesions; 1 = focal demyelinative lesions; 2 = diffuse demyelinative lesions).

To investigate the possible relation between the correction parameters and the incidence of demyelination in individual rats, the neuropathological score of each rat was plotted as a function of both the maximal rate of correction and the increase in plasma [Na^+] over the first 24 hours of correction (Fig. 3). These two correction parameters were chosen because they were found to be most highly correlated with the presence of demyelinative lesions in the corrected rats (Table 2). Animals bearing focal and diffuse neuropathological lesions had in common either high maximal correction rates and/or high 24-hour correction magnitudes. Analysis of the 24-hour correction magnitude alone indicated an absolute threshold for producing demyelinative lesions of 16 mmol/liter; no animals corrected by an amount less than this at 24 hours developed demyelination, in comparison to a 42% incidence of demyelination in rats corrected at 24 hours by amounts equal to or greater than this. Similarly, analysis of the maximum correction rate alone indicated an absolute threshold for producing demyelinative lesions of 1.75 mmol/liter · hr; no animals corrected at a maximal rate less than this developed demyelination, in comparison to a 54% incidence of demyelination in rats corrected at maximal rates equal to or greater than this. However, inspection of Figure 3 shows that the rats who developed demyelinative lesions at the lower 24-hour correction magnitudes all had relatively high maximum correction rates, and similarly the rats who developed demyelinative lesions at lower maximal correction rates all had relatively high 24-hour correction magnitudes. Consequently, the same analysis was done using both of these parameters together, and this revealed that no rat developed demyelinative changes in whom both of two conditions were met: the maximal rate of correction of plasma [Na^+] was $\leq$4 mmol/liter · hr, and the magnitude of correction of plasma [Na^+] in the first 24 hours was $\leq$25 mmol/liter. In contrast, for all rats who exceeded *either* one of these two limits the incidence of demyelinative lesions was 64%.

Application of discriminant function analysis to these results (combining the rats with focal and diffuse lesions into a single demyelination group) showed that use of the maximum rate of correction correctly predicted the occurrence of demyelination in 58 of 67 rats (87%), while use of the magnitude of correction in the first 24 hours correctly predicted the occurrence of demyelination in 44 of 67 rats (67%). Nonetheless, it should be noted that a number of rats survived fairly rapid and relatively large corrections without subsequently developing brain demyelinative lesions (Fig. 3).

Discussion

The data presented in this paper is consistent with the results of multiple previous experimental studies demonstrating that correction of hyponatremia is associated with the development of neurological dysfunction and brain myelinolysis in animals [11–16]. Although this phenomenon is now generally accepted, our results extend the existing data base in this area because of the methodology utilized in our studies, which differs significantly from previous studies in this area in a number of aspects.

In contrast to previous studies which employed relatively short periods of acute hyponatremia, our studies focused on animals with chronic long-standing hyponatremia. Because both clinical [4] and animal [14, 20] studies have suggested that chronicity of hyponatremia increases the risk of brain myelinolysis following subsequent correction, this model should have maximally enhanced sensitivity for detecting demyelination in response to changes in plasma [Na^+]. An additional advantage is that rats adapted to chronic hyponatremia using this model experience virtually no mortality, and little observable morbidity, despite long periods of severe hyponatremia [17]. Thus, unlike the high mortality rates even before correction of hyponatremia characteristic of many [12–14, 16], though not all [11, 15], previous studies, using this model it is clear that the functional neurological deficits following correction of the hyponatremia were causally related to the correction, rather than a result of the preceding hyponatremia itself. The most likely reason for both of these differences is the brain volume regulation that occurs over time in response to the hyponatremia. Previous studies using this model have shown that after three weeks of chronic hyponatremia brain water contents completely return to normonatremic levels [17]. This absence of residual brain edema undoubtedly contributes to the low mortality and morbidity which is characteristic of the chronically hyponatremic rats. However, previous studies in rats have also demonstrated that brains of hyponatremic rats lose more water following increases in plasma osmolality than do brains of normonatremic rats [7, 18]. Thus, the same volume regulatory adaptation that allows animals to survive in the face of severe hyponatremia in some ways becomes maladaptive when the hyponatremia is corrected, by putting animals at risk for greater potential degrees of osmotic brain dehydration. Although a causal relation between osmotic dehydration and myelinolysis following correction of hyponatremia remains to be proven, it seems likely that this factor contributes in some way to the neuropathological abnormalities found [3, 15, 18].

Perhaps most importantly, our studies employed carefully monitored corrections of hyponatremia using methods analogous to those encountered clinically. Particularly worrisome in this regard has been the frequent use of intraperitoneal injections of hypertonic saline to correct hyponatremia in rats [11, 13, 15, 16]. This method leads to rapid increases in plasma [Na^+] due to prompt re-equilibration of the administered fluid throughout the extracellular space [21]. Such rapid increases in plasma [Na^+] so greatly exceed the rates with which hyponatremia is corrected clinically that their relevance to human disease is debatable. Although several previous studies have also employed methods of correction similar to ours based upon water restriction [11], infusion of hypertonic saline [12], and water diuresis [15], the absence of frequent monitoring of plasma [Na^+] during the corrections in these studies precluded a careful analysis of the relation between the maximal rate of the correction and the production of brain myelinolysis. Frequent monitoring of plasma [Na^+] allowed us to perform a more rigorous analysis of the relation between the various parameters characterizing the correction and the subsequent development of neuropathological changes. Figure 1 and Table 1 graphically illustrate the problem of calculating correction rates by averaging the starting and ending plasma [Na^+] over prolonged periods. The averaged 48-hour correction rates of all three treatment groups were virtually identical, and bore little relation to the neuropathology produced. Only correction rates that accurately reflected the more acute changes occurring during the first 24 hours of the correction were strongly correlated with the subsequent development of demyelinative lesions (Table 2).

In view of the above considerations, several conclusions can be drawn from our results. First, it is clear that severe hyponatremia by itself does not lead to the demyelinative changes characteristic of pontine and extrapontine myelinolysis. These findings are in agreement with several other studies that have examined this issue [13, 15]. However, because previous studies utilized shorter periods of hyponatremia, the possibility still existed that more chronic durations of sustained hyponatremia might cause such changes. The absence of any demyelinative changes in the brains of rats that were maintained hyponatremic for four weeks in this study now definitively eliminates this possibility as well.

Second, the analysis shown in Figure 3 strongly suggests that both the rate and magnitude of correction are important factors involved with the development of brain myelinolysis. Brain pathology clearly occurred in many rats that had high rates of correction but relatively small magnitudes of correction in the first 24 hours, and conversely demyelination was found in a few rats whose maximal rate of correction was relatively low but whose magnitude of correction in the first 24 hours was large. The maximal correction rate associated with demyelination in experimental animals has never been carefully defined previously, and in these studies it is particularly noteworthy that this factor represented the single best predictor of subsequent development of demyelination. Myelinolysis was first observed in rats whose maximal rate of correction exceeded 1.75 mmol/liter · hr, and the incidence of demyelination increased progressively with more rapid rates of correction. However, at lower 24-hour magnitudes of correction (≤25 mmol/liter) the threshold for producing myelinolysis appeared to be closer to 4 mmol/liter · hr (Fig. 3). The 24 hour magnitude of correction associated with demyelination in these studies is similar to results reported in several earlier studies. Myelinolysis was first observed in rats whose 24 hour magnitude of correction exceeded 16 mmol/liter, a value analogous to that suggested in previous studies of rats [20] and dogs [12]. However, at lower maximal correction rates (≤4 mmol/liter · hr) the threshold for producing myelinolysis appeared to be closer to a 24 hour magnitude of 25 mmol/liter (Fig. 3), a value very similar to that suggested by more recent studies in rats [16] (although some concerns have been raised about the methodology used in this previous study [22]).

Although the incidence of demyelination was 64% in those rats whose maximal correction rate exceeded 4 mmol/liter · hr or whose total magnitude of correction in the first 24 hours exceeded 25 mmol/liter, it is striking that not all such rats developed myelinolysis. The absence of demyelination in some rats that experienced just as rapid and as large corrections as rats that developed diffuse myelinolysis indicates that a rapid and/or large correction of hyponatremia represents a very significant risk factor for the development of neuropathological changes, but does not obligatorily lead to such changes in all cases. Nonetheless, Figure 3 indicates that the degree of risk increases as both of these parameters increase, and is especially pronounced when high maximal rates and large 24 hour magnitudes are both present.

As with any experimental study, many potential qualifications of the above conclusions must be taken into account and alternative interpretations considered. The first issue is the degree to which the demyelinative lesions observed were related to functional neurological deficits. Although our data only demonstrate associations and cannot prove a causal relation between these effects, the strong association between a variety of neurological findings, including impaired ingestive behaviors, and the presence of brain demyelinative lesions (Fig. 2, Tables 2 to 4) strongly supports the functional significance of the brain pathology produced. These results also indicate that such impairments in ingestive behaviors represent an easily quantifiable indicator of the likely presence of diffuse myelinolysis following correction of hyponatremia in rats for future studies. Nonetheless, the presence of demyelinative lesions in a large portion of totally asymptomatic rats emphasizes that the absence of overt neurological symptoms cannot be interpreted as indicative of an absence of brain damage following correction; perhaps finer functional testing might have uncovered more subtle neurological deficits in these animals.

A second potential qualification is that these studies address chronic hyponatremia rather than acute hyponatremia, and the latter situation appears to be less prone to the development of brain myelinolysis following correction of the hyponatremia [4, 8, 14, 21]. Because of this, it has been argued that chronic asymptomatic hyponatremia is not an ideal, or possibly even appropriate, model in which to study the neurological and neuropathological sequelae of correction of hyponatremia [16] since clinically asymptomatic hyponatremic patients do not require as prompt a correction [13]. Although this argument has obvious validity, the advantage of using a model of chronic asymptomatic hyponatremia is that this should represent the worst possible case for the development of demyelinative lesions following correction of the hyponatremia. Because clinically it is often uncertain what the duration of hyponatremia has been in any specific patient, and because many potential early hyponatremic symptoms are relatively nonspecific (for example, nausea, headache, and confusion), the distinction between acutely symptomatic and chronically asymptomatic hyponatremic patients is not always as clear as would be desirable. Consequently, any correction parameters found to be safe in chronic asymptomatic hyponatremia should in turn be safe for correcting hyponatremia of any duration, even for those cases in which the actual duration is not known. Nonetheless, it must be acknowledged that several previous studies have clearly shown that animals with acute hyponatremia of shorter durations can easily tolerate more rapid corrections with less morbidity and mortality than found using this model of chronic hyponatremia [15, 20].

A third potential qualification relates to the severity of the preexisting hyponatremia prior to correction. Animals in this study were severely hyponatremic (plasma $[Na^+] = 112 \pm 1$ mmol/liter), but obviously lower plasma $[Na^+]$ levels have been achieved in both patients and experimental animal studies. Most clinical cases of myelinolysis have in fact been reported in patients with plasma $[Na^+]$ levels ≤105 mmol/liter prior to correction [4, 23]. Whether the thresholds described here would also be true of rats with more severe degrees of chronic hyponatremia is unknown, but since brain electrolyte losses appear to be proportional to the degree of hyponatremia induced, presumably more severely hyponatremic animals would experience greater degrees of osmotic dehydration following equivalent corrections of their hyponatremia. Whether this would in turn result in the production of brain myelinolysis at

even lower maximal rates and 24 hour magnitudes of correction remains to be evaluated by future studies.

Several final qualifications address specific aspects of the methodology employed in these studies. The protocol used to induce and maintain chronic hyponatremia in these studies utilized DDAVP rather than AVP, which is of course the physiological antidiuretic hormone. Although AVP works equally well in this experimental paradigm [19], DDAVP was used because it is a selective V_2 receptor agonist and its use therefore avoids potential confounding vascular and hepatic effects associated with AVP infusions. Since clinical hyponatremic states, including most cases of the syndrome of inappropriate antidiuresis, are generally associated with plasma AVP levels below the threshold for significant vasopressor effects [24], the use of DDAVP in animal models is advantageous since it enables the achievement of sufficient degrees of antidiuresis to maintain severe hyponatremia but without any risk of producing unwanted vascular effects. Higher infusion rates of AVP also frequently cause catabolism and weight loss, which may represent a contributory factor to the higher mortality rates observed in some previous hyponatremic models [12–14, 16] as compared to the very low morbidity and mortality observed in this model [17]. Nonetheless, the use of DDAVP rather than AVP must be acknowledged as a potentially significant difference between this model and human pathophysiology.

Another important methodological consideration concerns the use of different correction methods to achieve the varied correction rates and magnitudes shown in Figures 1 and 3. Although most of the demyelination occurred in the rats who had much higher maximal rates of correction, because most of these rats were corrected with hypertonic saline infusions, it remains possible that the method of correction in some ways also contributed to the observed neuropathological sequelae. Hypertonic saline infusion produces a volume expansion, in contrast to the volume contraction produced in the rats corrected by water diuresis and water restriction. This further induced hypervolemia in animals who are already volume-expanded by retained water [25] could conceivably either worsen, or ameliorate, brain osmotic dehydration in response to the increased ECF osmolality through a variety of possible mechanisms such as systemic hypertension, interstitial edema, or altered vascular resistance. Neither previous studies nor the present results enable a critical evaluation of this possibility, and future studies comparing demyelination in groups of animals that achieve a spectrum of different maximal correction rates and magnitudes using the same correction methods will be necessary to address this question. Until such studies are done, however, the most likely explanation for the high rate of myelinolysis in the rats corrected with hypertonic saline remains the very rapid rate of rise of plasma $[Na^+]$ which was purposely induced in this group.

Finally, although our results strongly suggest that both the rate and magnitude of correction are important risk factors for the development of brain myelinolysis in chronically hyponatremic rats, it should be recognized that this conclusion is based on a retrospective analysis of the results. The actual design of the study involved using different methods of correction to produce a spectrum of different correction rates. Although a spectrum of different correction magnitudes also was produced, in these studies the maximal rate and magnitude of increases in plasma $[Na^+]$ were intercorrelated to the extent that approximately 25% of the variance of each of these two parameters could be explained by changes in the other. Consequently, future studies in which the magnitudes of increases in plasma $[Na^+]$ are varied independently of changes in the rate of increase will be necessary to definitively address the independent contribution of the absolute magnitude of correction of plasma $[Na^+]$ to brain myelinolysis in experimental hyponatremia.

Because these studies were done in an effort to better understand the factors associated with the development of pontine and extrapontine myelinolysis in human patients, it is appropriate to attempt to place our results into a clinical perspective, as has been done in many previous reports of experimental studies [11–16]. However, it would obviously be inappropriate to suggest that the limits described for safely correcting hyponatremia in rats or any other species apply to humans as well, in either this or other experimental studies. The clinical relevance of these studies does not lie in the numerical findings, but rather in the clear demonstration that it is possible to ascertain safe limits for both the rate and magnitude of correction of hyponatremia that eliminate, or at least minimize, the risk of precipitating brain myelinolysis. The exact values for these limits in human patients can only be ascertained by clinical studies, many of which have already been done and describe correction limits not dissimilar to those found in these experimental studies [3–5, 8, 26, 27].

An additional important implication of these studies is that they demonstrate the similarity of experimental studies in hyponatremic animals to human disease in hyponatremic patients. Our data would suggest that the high incidence of brain demyelination in some earlier animal studies, and particularly those employing intraperitoneal injections of hypertonic saline, might be in part due to the inadvertent production of very rapid correction rates. When these rates were carefully monitored, corrections in experimental animals that remained within ranges usually accomplished in clinical settings were not often accompanied by brain myelinolysis, whereas those that far exceeded typical clinical ranges frequently were associated with adverse neurological findings and brain myelinolysis. Thus, we would suggest that the relative rarity of reports of this disorder in human patients is not really a reflection of basic differences between human and animal physiology, but rather is indicative of a similarity across species provided that experimental studies are done in a way that more closely resembles human disease and therapy.

In summary, we evaluated the incidence of neurological and neuropathological findings following correction of hyponatremia at different rates in chronically hyponatremic rats. Brain myelinolysis was first observed in animals whose maximal (4 hr) rate of correction exceeded 1.75 mmol/liter · hr, and the incidence of demyelination increased progressively with more rapid rates of correction. The occurrence of myelinolysis was found to be related to the magnitude of correction as well, and was first observed in rats whose absolute increase in plasma $[Na^+]$ at 24 hours exceeded 16 mmol/liter and also increased greater following larger 24 hour magnitudes of correction. However, analysis of the incidence of myelinolysis using both of these correction parameters together indicated that demyelination did not occur in rats whose maximal rate of increase in

plasma [Na^+] did not exceed 4 mmol/liter · hr *and* whose absolute increase in plasma [Na^+] did not exceed 25 mmol/liter in the first 24 hours; for all rats who exceeded *either* one of these two limits the incidence of demyelinative lesions was 64%. Our studies therefore suggest that both the rate and the magnitude of correction of hyponatremia are important variables in producing myelinolysis in experimental hyponatremia. However, additional studies in which each of these two related factors are varied independently of the other will be necessary to determine more precisely how "rate" and "magnitude" interact to increase the risk of brain myelinolysis.

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