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PROTEIN OXIDATION AND MYELINOLYSIS OCCUR IN BRAIN FOLLOWING RAPID CORRECTION OF HYPONATREMIA

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Myelinolysis occurs following rapid correction of hyponatremia in both humans and experimental animals. Although the mechanism of this effect at present is unknown, we have examined the possibility that a rapid rise in serum sodium following hyponatremia potentiates an oxidative stress and results in the oxidation of cellular proteins. In these studies, rats treated with 1 M NaCl following 3 days of vasopressin-induced hyponatremia exhibited myelinolysis in the corpus striatum and thalamus as well as significant increases in soluble oxidized proteins in the brain. These changes did not occur in rats treated with 0.155 M (0.9%) NaCl following 3 days of hyponatremia.

Because severe hyponatremia constitutes a medical emergency that may result in seizures, cerebral edema, and death, there is a strong temptation to correct it rapidly. However, the rapid correction of severe hyponatremia with hypertonic saline has been shown to result in myelinolysis in the rat (1,2), rabbit (3), and dog (4), and it may be associated with the damage to myelin in central pontine myelinolysis in man (1-5). Moreover, myelin damage occurring in similar areas of the brain following hyperoxic reperfusion of ischemic Mongolian gerbil brain (6) is associated with peroxidation of omega-6 fatty acids (7), suggesting that conditions associated with myelin damage may also be associated with oxidative stress. Although considerable evidence indicates lipid peroxidation is important in tissue damage, little attention has been focused on the possible role of protein oxidation in this process. Inactivation of enzymes resulting from oxidation of proteins may be a major biochemical mechanism of cellular damage in oxidative stress. In the

Abbreviation: DNPH, 2,4-dinitrophenylhydrazine.

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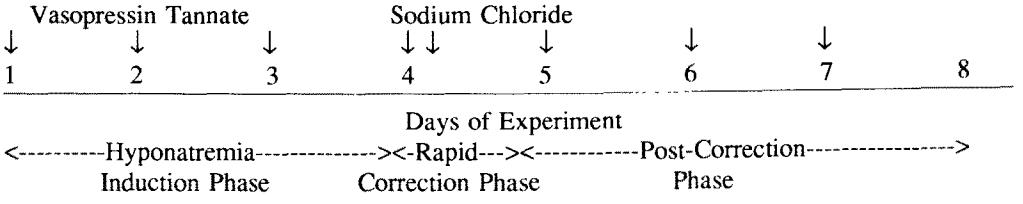
studies presented here, using a model of electrolyte–induced myelinolysis in the rat, we have demonstrated that soluble brain proteins are oxidized following rapid correction of hyponatremia.

MATERIALS AND METHODS

Animal Model System. Experiments were performed on 105 male Sprague- Dawley rats (Taconic Farms), aged 5–7 months and weighing 480.0 ± 5.0 gram (mean ± S.E.M., range 370–600 gram). Animal weight was similar for each experimental group. Hyponatremia was induced by 3 daily subcutaneous injections of 0.55 units vasopressin tannate (Parke-Davis)/100 gram body weight. Food was restricted throughout the experiment. On day 4, 1 ml blood was obtained from the tail vein for serum Na^+ concentration, and then rapid correction of hyponatremia was achieved by making a single intraperitoneal injection of 1 M NaCl (2.1 ml/100 gram body weight). In a subset of 5 animals, 1 ml 1 M KCl/100 gram body weight was given via gastric tube at the same time. In a second subset of 3 animals, 0.155 M (0.9%) NaCl (2.1 ml/100 gram body weight) was substituted for 1 M NaCl. Four to five hours after intraperitoneal injection, 0.155 M NaCl (6.7 ml/100 gram body weight) was given subcutaneously to all animals. On days 5, 6, and 7, subcutaneous injection of 0.155 M NaCl was given equal to the loss of body wt from the previous day. The mean weight losses per day ± S.E.M. were: Day 5 = 49.5 ± 2.2 gram; Day 6 = 38.4 ± 2.2 gram; and Day 7 = 19.6 ± 2.2 gram. Procedures were done under (15–35 mg) ketamine HCl (Parke–Davis) anesthesia.



Animals were sacrificed following overdose (300–450 mg) of ketamine HCl by day 8 (day 4 post-correction). Animals were quickly exsanguinated by aspiration of blood (8–12 ml) from the right atrium. Serum was separated after clotting and centrifugation. The brain was removed within 3 minutes of exsanguination and frozen at −70°C.

Preparation of Brain Extracts. Brain samples were sonicated in 10 mM Hepes (Fluka) and 1.1 mM ethylenediamine tetraacetic acid (Fisher) buffer, pH 7.4, with the following protease inhibitors (Boehringer–Mannheim): 0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin, 0.7 μg/ml pepstatin, and 40 μg/ml phenylmethylsulfonyl fluoride. The crude extract preparation was centrifuged at 100,000 x g at 0°C for 5 min in 1.0 to 1.5 ml volumes. The supernatant was recovered and the protein concentration was determined using the Pierce–BCA assay (8). The protein concentrations were adjusted to 0.6–0.8 mg/ml.

The level of soluble brain protein oxidation was determined by reacting proteins with a carbonyl-specific reagent, 2,4–dinitrophenylhydrazine (Eastman Kodak), according to the method previously described (9). The samples were precipitated with an equal volume of 20% trichloroacetic acid and then treated with either 0.2% DNPH in 2 N HCl or with 2 N HCl alone. The samples were incubated for 1 hr at 24°C with stirring at 5 min intervals. Samples were reprecipitated with an equal volume of 20% trichloroacetic acid, loaded onto Whatman 3 MM filters, and sequentially extracted with ethanol:ethyl acetate (V;V, 1:1) and with 10% trichloroacetic acid. Filters were placed in 30 ml beakers, the protein eluted with 2 ml 6 M guanidine HCl, pH 6.0, for 2 hr at 24°C, and the protein solutions were then centrifuged at 5000 x g for 15 min at 4°C to remove insoluble debris. The difference spectrum of the DNPH–treated

sample versus the sample treated with HCl alone was performed using a Hewlett–Packard 8452 diode array spectrophotometer. The 2,4-dinitrophenylhydrazine incorporated into the protein was calculated upon an absorptivity of 21,000 M^{-1} cm^{-1} for most aliphatic hydrazones (10).

Assays. Chemical analyses on serum were obtained from Metpath, Inc., Rockville, MD. Sodium and potassium concentrations were determined by the ion specific electrode method (11).

Morphology. Animals examined morphologically (n = 19) were perfused immediately through the left ventricle with the descending aorta clamped, following exsanguination, with 1 liter of 4% paraformaldehyde (E.M. Grade, Polysciences, Warrington, PA) in 0.1 M sodium phosphate buffer, pH 7.4. Brains were sliced with the 3 mm Jacobowitz rat brain slicer (Zivic–Miller) and embedded in paraffin. Adjacent sections were stained with haematoxylin and eosin, Luxol fast blue/cresyl violet for myelin, according to the Bodian method for axons, and immunocytochemically according to the avidin–biotin–immunoperoxidase complex method (12) (Vector Laboratories, Burlingame, CA). The primary antibodies and incubation conditions were: a) 1:1500 rabbit anti–bovine myelin basic protein (Chemicon International, Los Angeles, CA) in 0.5 M Tris buffer, pH 7.6, (13) for 45 min. b) 1:200 rabbit anti–bovine glial fibrillary acidic protein (Dako Corporation, Santa Barbara, CA) in phosphate–buffered saline, pH 7.4, for 1 hr.

RESULTS

Hyponatremia. The results in Figure 1 show that vasopressin tannate and 2.5% dextrose in water induced a decrement in serum sodium to 105.6 ± 1.1 mM (mean ± S.E.M., n = 61) by day 4, returning after rapid correction with hypertonic saline to normal by day 6. Serum potassium concentration remained unchanged throughout. During induction of hyponatremia, all animals became less active and responded more weakly. After hypertonic saline was given, most animals became more active (3–6 hr), but later became lethargic. By days 5 and 6 (post–correction phase), the animals had ruffled fur, ataxic gait, and diminished extension of the hind legs on walking or standing.

Protein Oxidation. The levels of oxidized soluble proteins in the brains of animals with severe hyponatremia were unchanged from normal controls (Figure 2). However, increase in oxidized proteins was evident by day 5. When 0.155 M NaCl was substituted for the 1 M NaCl intraperitoneal injection, the sodium concentration increased by 17 mM over 24 hr, but no increase in oxidized proteins was observed (5.13 ± 0.79 (S.E.M.) nmol DNPH /mg protein). Three animals treated with 1 mmol KCl/100 gram body weight showed comparable oxidation of brain proteins to animals not receiving KCl (8.83 ± 0.18 (S.E.M.) nmol DNPH/mg protein).

Morphology. Lesions of myelin sheaths were found symmetrically within the corpus striatum of all animals by the first day post–correction (Figure 3). These striatal lesions were characterized by loss of oligodendrocytes, fragmentation and swelling of myelin sheaths, and infiltration of macrophages. Many well–preserved neuronal cell bodies were found within the areas of myelin damage. Pairs of astrocytes, with immunoreactivity to glial fibrillary acidic protein, were seen along the borders of the lesions. Similar pairs of cells, with no

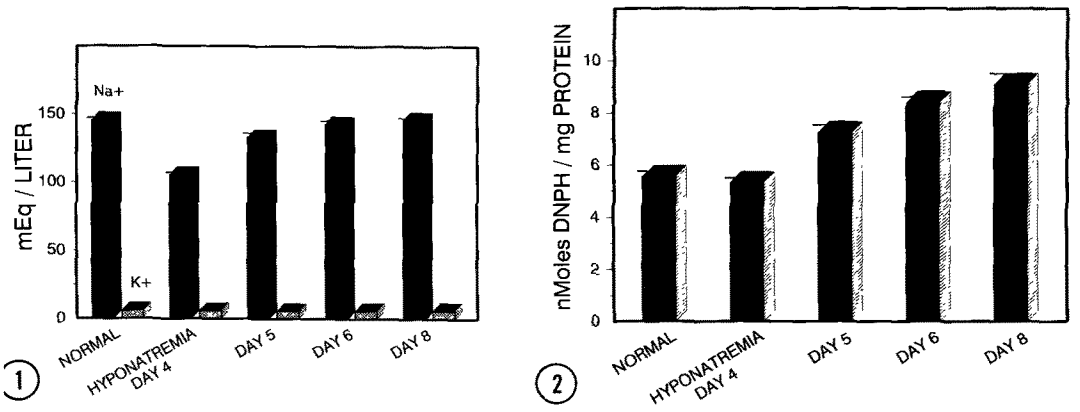


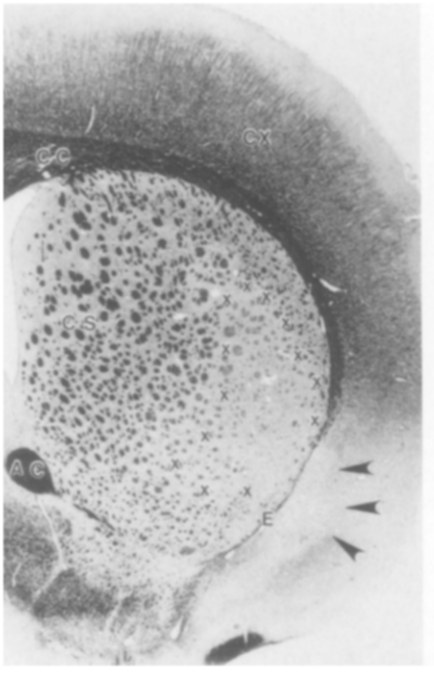
FIGURE 1. SERUM SODIUM AND POTASSIUM CONCENTRATIONS. Hyponatremia was induced by 3 daily treatments with vasopressin tannate and 0.138 M (2.5%) dextrose in water and corrected by treatment with 1 M NaCl on day 4. Serum sodium values are represented by solid bars and serum potassium are represented by cross-hatched bars. After induction of hyponatremia, the level of serum sodium (105.6 ± 1.1 meq/L, n=61) is significantly less than both the normal control value (n=18, P <0.000001) and the first day of the post-correction phase (n=20, P <0.002). Serum potassium concentration remained essentially unchanged during hyponatremia and its correction. Probability values were determined by one-tailed ANOVA.

FIGURE 2. OXIDIZED SOLUBLE PROTEINS IN THE BRAIN. Oxidized soluble brain proteins were assayed as described in Materials and Methods. Results are expressed as nanomoles of DNPH reacting per milligram protein. All post-correction phase values are significantly higher than either normal control values, 5.56 ± 0.13, or hyponatremic values 5.33 ± 0.26 (S.E.M.). Probability values are given first calculated by one-tailed ANOVA and second by the Wilcoxon Rank test. The values for day 5 (n=5), 7.25 ± 0.78 (S.E.M.), were P <0.04, P <0.004, day 6 (n=3), 8.43 ± 0.18 (S.E.M.), P < 0.000005, P <0.02, and day 8 (n=4), 9.10 ± 0.97 (S.E.M.), were P <0.002, P <0.01, all compared to normal values.

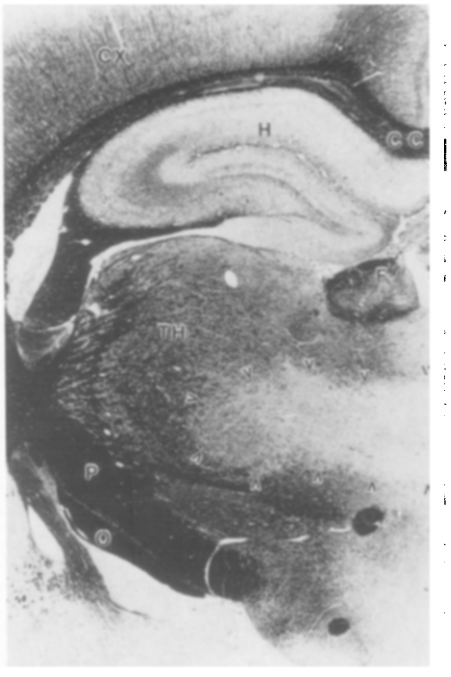
immunoreactivity to glial fibrillary acidic protein, were seen within the lesions. Myelin lesions were seen within the thalamus (Figure 4) and brain stem tegmentum in some animals. No difference was found in the brains of animals treated with 1 mmol KCl/100 gram body weight.

DISCUSSION

In this study, rapid correction of severe hyponatremia is shown to be associated with both myelinolysis and oxidation of soluble brain proteins. These findings are consistent with the hypothesis that oxidative damage involving both lipids and proteins may be correlated with demyelination (14–16). *In vitro* studies have shown the brain to be highly susceptible to autoxidation (17) and myelin proteins to be damaged by exposure to H_2O_2 and Cu^{++} (18). In this study, the decrease in immunoreactivity to myelin basic protein and glial fibrillary acidic protein in areas of myelin damage may be the result of protein oxidation and subsequent selective degradation by neutral proteases (19,20).



(3)



(4)

FIGURE 3. MYELIN DAMAGE IN CORPUS STRIATUM FOUR DAYS POST-CORRECTION. Myelin lesions were observed in light micrographs (x22.5) of lateral corpus striatum (CS) of rat brains four days after rapid correction of severe hyponatremia. Imunoperoxidase staining for myelin basic protein is described in Materials and Methods. In the corpus striatum (CS), the area of reduced staining to myelin basic protein antiserum is outlined by x's and in the adjacent cerebral cortex by larger arrow heads (>'s) The lesion extends across the external capsule (E) into subcortical white matter. Other areas of the cerebral cortex (CX), corpus callosum (CC), and anterior commissure (AC) are unaffected.

FIGURE 4. MYELIN DAMAGE TO THALAMUS FOUR DAYS POST- CORRECTION. Myelin lesions were observed in the thalamus (TH) of rat brains (x22.5) four days after rapid correction of hyponatremia. The lesion (outlined by small >'s) shows an area within the thalamus of reduced staining to myelin basic protein antiserum. No apparent lesion is seen in the fornix (F), hippocampus (H), corpus callosum (CC), posterior limb of the internal capsule (P), or optic tract (O).

Increased oxidation of soluble brain proteins following rapid correction of hyponatremia is likely an indicator of oxidative stress in the brain. In addition, oxidized myelin proteins may possibly provide a chemotactic stimulus capable of recruiting activated macrophages to damaged myelin sheaths and providing additional oxidative damage by oxygen free radicals.

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