Protective effect of dexamethasone on osmotic-induced demyelination in rats

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Abstract

Central pontine myelinolysis (CPM) is a serious demyelination disease commonly associated with the rapid correction of hyponatremia. Although its pathogenesis remains unclear, the disruption of the blood–brain barrier (BBB) as a consequence of a rapid increase in serum sodium concentration is considered to play a critical role. Since glucocorticoids are known to influence BBB permeability and prevent its disruption as a result of hypertension or hyperosmolarity, we investigated whether dexamethasone (DEX) could protect against osmotic demyelination in an animal model of CPM. Hyponatremia was induced in rats by liquid diet feeding and dDAVP infusion. Seven days later, the animals' hyponatremia was rapidly corrected by injecting a bolus of hypertonic saline intraperitoneally. Rats subjected to this treatment displayed serious neurological impairment and 77% died within 5 days of rapid correction of their hyponatremia; demyelinative lesions were observed in various brain regions in these animals. On the other hand, rats that were treated with DEX (2 mg/kg, 0 and 6 h after hypertonic saline injection) exhibited minimal neurological impairment and all were alive after 5 days. Demyelinative lesions were rarely seen in the brains of DEX-treated rats. A marked extravasation of endogenous IgG was observed in the demyelinative lesions in the brains of rats that did not receive DEX, indicating disruption of the BBB, but was not observed in DEX-treated rats. Furthermore, Evans blue injection revealed a significant reduction in staining in the brains of DEX-treated rats ($P < 0.05$). These results indicate that early DEX treatment can prevent the BBB disruption that is caused by the rapid correction of hyponatremia and its associative demyelinative changes, and suggest that DEX might be effective in preventing CPM.

Introduction

Central pontine myelinolysis (CPM) is a serious and often fatal human demyelination disease commonly associated with an overly rapid correction of hyponatremia (Rojiani et al., 1994), especially in patients with long-term hyponatremia (Adler et al., 1995; Baker et al., 2000). There are currently no guidelines concerning the appropriate rate at which such correction should be carried out in order to minimize the patient's risk of developing CPM. In addition to such guidelines, there is also a need for a protocol that could be used to prevent CPM if the correction of hyponatremia was too rapid. Although the pathogenesis of CPM remains unclear, accumulating evidence suggests that it occurs, at least in part, as a result of disruption of the blood–brain barrier (BBB) as a consequence of a rapid increase in serum sodium concentration. The brain adapts to chronic hyponatremia and limits brain swelling by extruding electrolytes and organic osmolytes (Soupart et al., 2002; Strange, 1992). The loss of brain solute during adaptation to hyponatremia lowers the osmotic set point for intracellular to extracellular water shifts following rapid correction of hyponatremia, resulting in cellular dehydration (Baker et al., 2000; Verbalis and

Gullans, 1993). Dehydration of brain vascular endothelial cells likely results in the shrinkage of these cells and disruption of their tight junctions (Adler et al., 1995). As a result, circulating immune factors such as complement proteins, lymphocytes, cytokines, and vasoactive amines gain ready access to CNS tissues and mediate oligodendroglial injury and demyelination (Baker et al., 2000).

It has been reported that glucocorticoids regulate BBB permeability (Hedley-Whyte and Hsu, 1986) and prevent BBB disruption induced by hypertension (Johansson and Linder, 1978) and hyperosmolality (Neuwelt et al., 1982). Recent studies demonstrated that glucocorticoids reinforce the BBB in vivo (Hoheisel et al., 1998; Sinton et al., 2000). These findings lead us to hypothesize that glucocorticoids might be effective in preventing BBB disruption following the rapid increase in serum sodium concentration and thus inhibit brain demyelination. In the present study, we examined whether dexamethasone (DEX) had a protective effect on osmotic demyelination using the rat CPM model.

Materials and methods

Animal experiments

Our experimental protocol is summarized in Fig. 1. Male Sprague–Dawley rats (body weight 250–300 g; Chubu Science Materials, Nagoya, Japan) were housed in a standard animal facility under conditions of constant temperature (23°C) and a 12-h/12-h light/dark cycle. Rats had ad lib access to standard chow and tap water until initiation of the protocol to induce hyponatremia. Rats were rendered hyponatremia using methods previously described
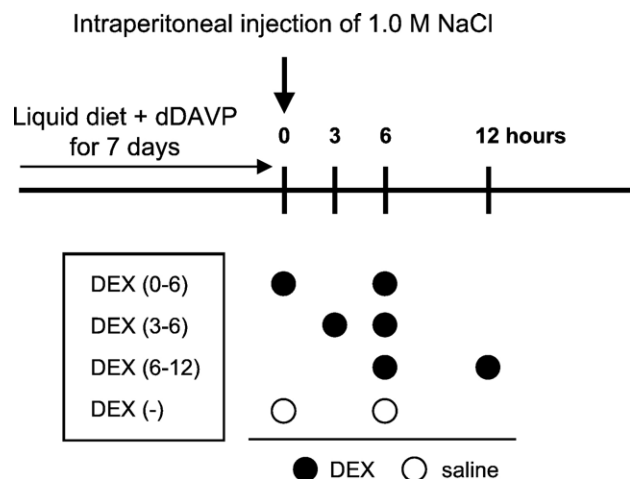

Fig. 1. Experimental protocol. Rats were rendered hyponatremic by liquid diet feeding and continuous dDAVP infusion for 7 days, after which their serum sodium level was rapidly corrected with an intraperitoneal injection of 1.0 M NaCl. The experimental groups were as follows: animals received an injection of DEX (2 mg/kg body weight) subcutaneously at either 0 h and 6 h (DEX (0–6) group), 3 h and 6 h (DEX (3–6) group), or 6 and 12 h (DEX (6–12) group) after hypertonic saline injection; control rats (DEX (−) group) were injected with saline 0 and 6 h after correction.

(Verbalis and Gullans, 1991). Briefly, osmotic minipumps (Alzet model 2002; Alza, Palo Alto, CA) containing the vasopressin V2 receptor agonist, 1-deamino-8-D-arginine vasopressin (dDAVP, 10 μg/ml; Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) were implanted subcutaneously under ether anesthesia, by which dDAVP was injected continuously at a rate of 5 ng/h. After 2 days of dDAVP administration, rats were water loaded by substituting their daily feedings with a liquid formula (Isocal plus; Mead-Johnson, Evansville, IN). Hyponatremia was induced by both dDAVP infusion and liquid diet feeding. Seven days after the start of liquid formula feeding, serum sodium levels were rapidly corrected by a bolus injection of 1.0 M NaCl (1.5 ml/100 g of body weight) administered intraperitoneally. DEX (Decadron, 2 mg/kg body weight; Merk and Co., Inc., Whitehouse Station, NJ) was subcutaneously injected at 0 h and 6 h [DEX (0–6) group], 3 h and 6 h [DEX (3–6) group], or 6 h and 12 h [DEX (6–12) group] after hypertonic saline injection. Control animals [DEX (−) group] were injected with an equivalent volume of saline. After the injection of hypertonic saline, the liquid diet was removed and each rat was given access to normal chow and water ad libitum. Neurological scores after the rapid correction of hyponatremia were assigned according to the following criteria as previously reported with minor modification (Baker et al., 2000): 6, no impairment; 5, poorly grooming; 4, slow or awkward gait; 3, limb weakness and/or paralysis; 2, seizures, severe motor deficits; 1, complete inability to move; 0, death. Blood was drawn via tail transection under light ether anesthesia from all animals and their serum sodium concentration was measured using an auto analyzer (Hitachi Ltd., Tokyo, Japan). All procedures were performed in accordance with institutional guidelines for animal care at Nagoya University, which conform to National Institutes of Health animal care guidelines.

Histological evaluation

All rats were sacrificed, under deep ether anesthesia, by transcardial perfusion with 4% paraformaldehyde in phosphate buffered saline (PBS) 4 days after the rapid correction of hyponatremia. Brains were removed, postfixed in 4% paraformaldehyde in PBS for 18 h at 4°C and were cryoprotected in 10%, 20%, and 30% sucrose in PBS at 4°C for 24 h each. Ten-micron sections were prepared using a cryostat. The sections were placed on glass slides and kept at −80°C until use.

Demyelinative lesions were evaluated by Luxol fast blue and cresyl violet staining. In addition, myelin immunocytochemical staining for myelin basic protein was performed to confirm the demyelinative lesions. Sections were incubated with PBS containing 0.3% Triton-X 100 (pH 7.4) for 20 min followed by a blocking buffer [10% bovine serum albumin (BSA) and 0.3% Triton-X 100 in PBS] for 60 min at 20°C. After washing with PBS, the sections were

incubated with anti-bovine myelin basic protein (Chemicon International, Inc., Temecula, CA) diluted at 1:10 with PBS for 24 h at 4°C. After incubation, sections were washed and incubated with biotin-conjugated anti-mouse IgG diluted at 1:250 with PBS for 3 h, followed by incubation with FITC-streptavidin diluted 1:100 with PBS. FITC-labeled sections were examined using a confocal microscope (Zeiss LSM 510; Carl Zeiss Inc., Oberkochen, Germany).

Immunostaining for IgG was performed to observe BBB disruption. Sections were immunostained with anti-rat IgG antibody as described previously (Baker et al., 2000) with minor modifications. Briefly, sections were incubated with PBS containing 0.3% Triton-X 100 for 20 min followed by a blocking buffer for 24 h at 20°C. After washing with PBS, the sections were incubated with biotin-conjugated anti-rat IgG diluted at 1:250 with PBS for 3 h, followed by incubation with FITC-streptavidin diluted 1:100 with PBS. FITC-labeled sections were examined using a confocal microscope (Zeiss LSM 510).

Determination of BBB permeability by Evans blue (EB) dye extravasation

EB is a diazo dye that binds to albumin in vivo and is used as a tracer for serum albumin. EB (2% in saline; 0.3 ml/100 g body weight) was injected intravenously under light ether anesthesia 6, 12, 24, or 48 h after the correction of hyponatremia by hypertonic saline injection. Thirty minutes after EB administration, the rats were perfused transcardially with saline to wash out the intravascular EB dye, under ether anesthesia. The perfusion was continued for 10 min until the fluid from the right atrium became colorless (Uyama et al., 1988). The brains were then removed and each hemisphere was quickly frozen for EB dye determination. EB was extracted from brain homogenates by precipitation with trichloroacetic acid and verified spectrophotometrically as previously described (Ovadia et al., 2001).

Statistical analysis

Results were expressed as the mean ± SE and statistical analyses were performed using a one-way ANOVA followed by Fisher's PLSD test. A Mann–Whitney non-parametric test was used to compare neurological scores. P values of less than 0.05 were considered to be significant.

Results

Serum sodium levels

Table 1 shows the changes in serum sodium concentration before and after hypertonic saline injection. Rats became severely hyponatremic by a combination of liquid diet feeding and continuous dDAVP infusion for 7 days, after which time, a bolus injection of hypertonic saline was

Table 1
Changes in serum sodium concentration following hypertonic saline infusion

| | Time after hypertonic saline injection | | | |
	0	6	24	(hour)
DEX (−)	99 ± 1	117 ± 2*	134 ± 3*	(mEq/l)
DEX (0–6)	100 ± 1	114 ± 1*	131 ± 1*	(mEq/l)

DEX (−) = without DEX; DEX (0–6) = DEX treatment 0 h and 6 h after hypertonic saline injection. Values are expressed as the mean ± SE (n = 5). There were no significant differences in serum sodium levels between the DEX (−) and DEX (0–6) groups at any time point.
* $P < 0.05$ vs. 0 h.

administered into the peritoneal cavity, rapidly increasing serum sodium levels by approximately 14 mEq/l in 6 h and 34 mEq/l in 24 h. DEX did not affect serum sodium levels after correction.

Neurological scores

The changes in neurological score after rapid correction of hyponatremia are shown in Fig. 2. Untreated rats that were rapidly corrected [DEX (−)] showed severe neurological impairments and 77% of them died within 5 days after correction. DEX treatment before and/or during the early phase of the correction improved the animals' neurological scores. When DEX was administered at 0 and 6 h after the hypertonic saline injection, all rats survived for at least 5 days after correction. Significant improvement was also seen when DEX was administered 3 and 6 h after hypertonic saline injection. However, DEX injection at 6 and 12 h failed to improve the animals' neurological scores.

Histological findings

Demyelinative lesions were observed in various brain areas 4 days after correction without DEX (see Fig. 3 for representative lesions); the lesions were clearly demarcated in the subcortical white matter (Fig. 3A). In addition, bilateral, symmetrical lesions were found in the vicinity of red nuclei (Fig. 3D). The neurons appeared to be relatively preserved in all of the demyelinative lesions. In the midbrain lesions, marked infiltration of microglia (cells with small blue nuclei stained with cresyl violet) was observed. Myelin immunocytochemical staining for myelin basic protein convincingly showed breakdown of myelin in the demyelinative lesion (Fig. 3G). No demyelinative lesions were apparent in the brains of DEX (0–6) (Figs. 3B, E) or uncorrected rat (Figs. 3C, F), suggesting that DEX treatment effectively prevented brain demyelination.

Prominent IgG immunostaining was observed in the subcortical white matter of DEX (−) rats (Fig. 4A). The fact that this staining was seen in the same regions as the demyelinative lesions suggested a causative effect of BBB
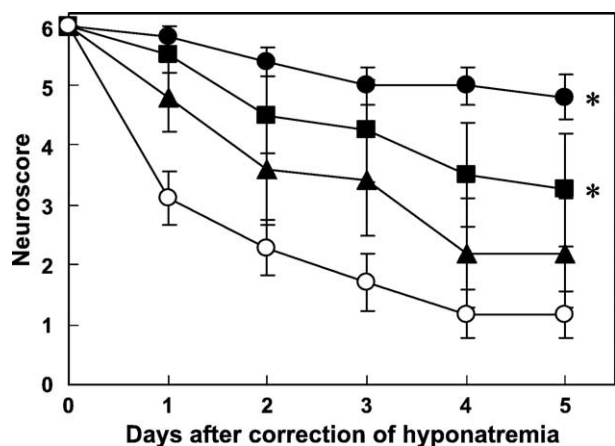
Fig. 2. Neurological scores after rapid correction of hyponatremia. Neurological symptoms were assessed using neurological scores as follows: 6, no impairment; 5, poorly grooming; 4, slow or awkward gait; 3, limb weakness and/or paralysis; 2, seizures, severe motor deficits; 1, complete inability to move; 0, death. DEX (−) group (○)(n = 17), DEX (0–6) group (●)(n = 5); DEX (3–6) group (■)(n = 5); DEX (6–12) group (▲) (n = 5). Values are expressed as the mean ± SE. *P < 0.05 compared to the DEX (−) group 5 days after the correction.

disruption in the pathogenesis of these lesions. No IgG staining was observed in the brains of DEX (0–6) (Fig. 4B) and uncorrected (Fig. 4C) rats.

BBB permeability after rapid correction

Along with serum sodium levels, BBB permeability, quantified by EB dye extravasation, also tended to increase after correction, with values showing significance 12 h after correction (Fig. 5A). Values peaked 24 h after correction and remained significantly elevated 48 h after correction (Fig. 5A). On the other hand, BBB permeability did not increase after correction in the DEX (0–6) group (Fig. 5B).

Discussion

It has previously been reported that demyelination occurred in rats whose magnitude of correction within 24 h exceeded 16 mEq/l (Verbalis and Martinez, 1991). In our study, we successfully induced osmotic demyelination in rats by rapidly correcting their hyponatremia by injecting a bolus of hypertonic saline, which increased their serum sodium concentration by approximately 34 mEq/l within 24 h. These rats subsequently developed serious neurological impairments including paralysis, seizures, and stupor. Rats that had not received DEX died within 5 days. In these animals, demyelinative lesions were observed in various
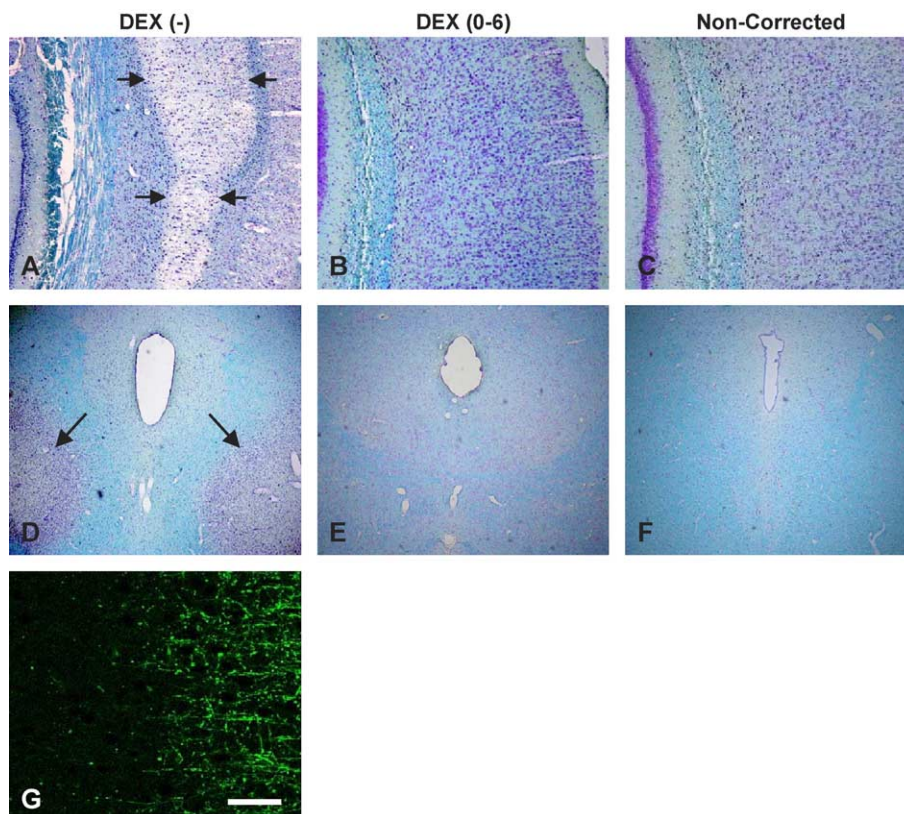

Fig. 3. Myelin staining. Brains that were removed 4 days after the rapid correction of hyponatremia were stained with Luxol fast blue and cresyl violet. A–C and D–F are sections of cerebral cortex and midbrain, respectively. DEX (0–6) rats were subcutaneously injected with DEX (2 mg/kg body weight) 0 and 6 h after hypertonic saline injection. The arrows indicate the presence of demyelinative lesions in the subcortical white matter and in the vicinity of red nucleus. (G) Higher magnification picture of myelin basic protein immunostaining of a demyelinative lesion in the cerebral cortex, showing the boundary between the myelinated (right) and demyelinated (left) region. Scale bar is 50 μm.
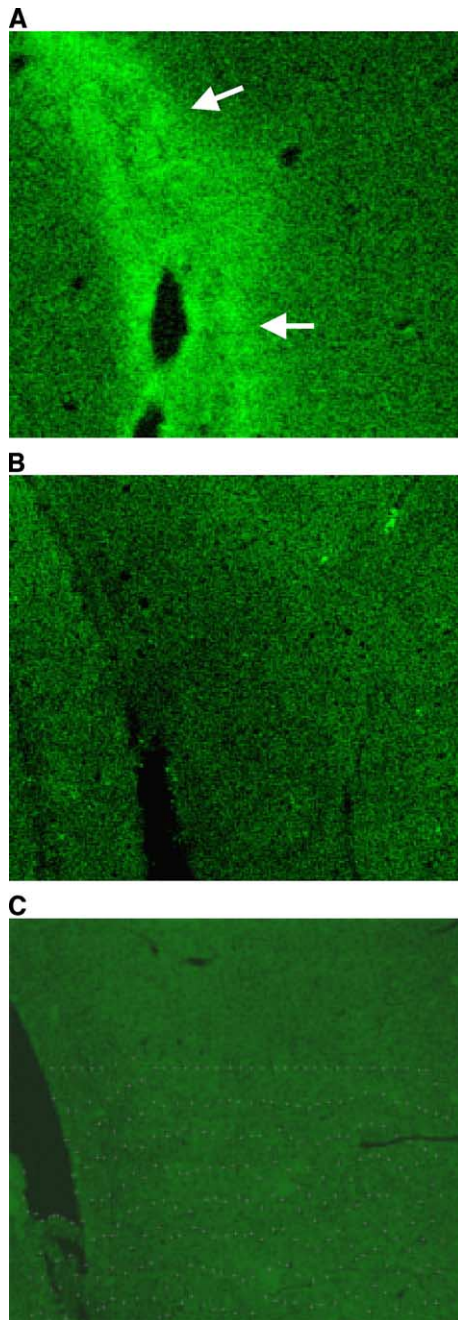
Fig. 4. IgG immunostaining of cerebral cortex. Brains were removed 4 days after the rapid correction of hyponatremia and immunostained for IgG. (A) DEX (−), (B) DEX (0–6), (C) uncorrected rat. The arrows indicate IgG staining in the subcortical white matter.

brain areas, especially the subcortical white matter and midbrain. Neurons in the demyelinative lesions appeared to be relatively well preserved in spite of the fact that some displayed significant microglial infiltration. These pathological changes were similar to those previously reported in this model (Kleinschmidt-DeMasters and Norenberg, 1982; Lien, 1995).

DEX treatment markedly improved the neurological scores and survival rates of rats after the rapid correction of

hyponatremia. Since there were no significant differences in serum sodium levels before and after correction between the DEX (−) and DEX (0–6) groups, the protective effects of DEX could not be attributed to changes in serum sodium levels. To examine whether DEX had any effect on the integrity of the BBB, brain sections were stained for IgG. Our data showed that the immunostaining for IgG overlapped with the locations of demyelinative lesions in the brains of DEX (−) rats, suggesting that BBB disruption played a critical role in the pathogenesis of osmotic demyelination. Lesions and IgG staining were rarely seen in DEX (0–6) rats, supporting the notion that the prevention of BBB disruption by DEX blocked brain demyelination in these animals.

DEX treatment was more effective when it was administered soon after correction. Specifically, DEX administration at 0 and 3 h, or 3 and 6 h after hypertonic saline injection had a markedly beneficial effect in preventing demyelination, while administration at 6 and 12 h after
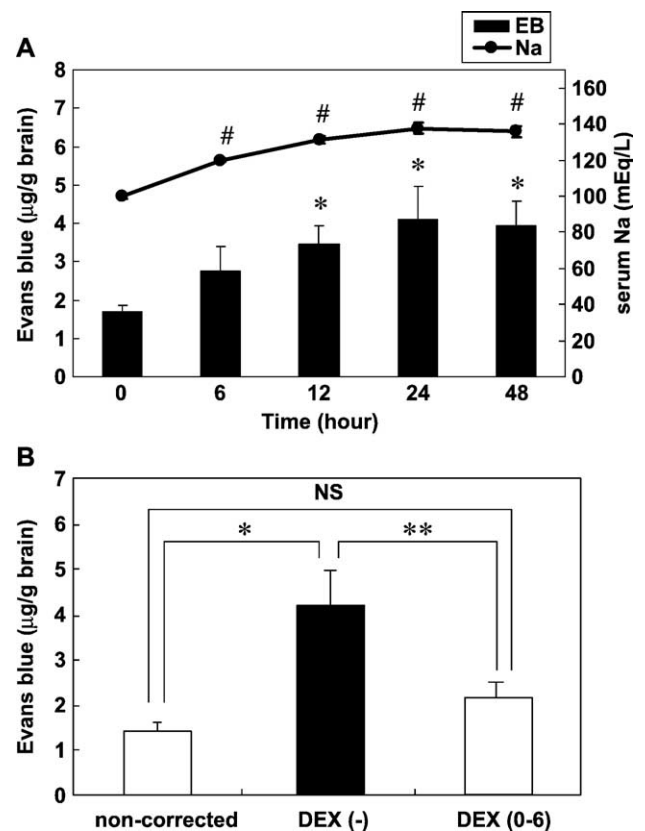

Fig. 5. BBB permeability after rapid correction of hyponatremia. (A)Time course of changes in BBB permeability and serum sodium levels after correction. Rats were rendered hyponatremic by liquid diet feeding and continuous dDAVP infusion for 7 days, after which their serum sodium level was rapidly corrected by the injection of hypertonic saline. Serum sodium levels were assessed 6, 12, 24, and 48 h after correction. BBB permeability was quantified using the Evans blue dye method 6, 12, 24, and 48 h after correction. Values are the mean ± SE ($n = 5$). $^{\#}P < 0.05$ vs. 0 h. (B) Effects of DEX on BBB permeability after rapid correction. BBB permeability was quantified 24 h after correction. DEX (2 mg/kg body weight) was subcutaneously injected 0 and 6 h after hypertonic saline injection. Values are the mean ± SE ($n = 5$). $^{*}P < 0.05$ compared to the uncorrected group. $^{**}P < 0.05$ compared to the DEX (−) group.

hypertonic saline injection had no effect. The fact that our data showed that BBB permeability tended to increase 6 h after correction suggests that DEX needs to be administered before that time in order for it to be effective in preventing demyelinative lesions. Once BBB disruption occurs, cytotoxic substances can permeate into the brain and damage oligodendroglia (Wren and Noble, 1989), which is difficult to reverse with DEX.

Though few reports suggested that DEX could prevent demyelination after the rapid correction of hyponatremia (Oh et al., 1990; Rojiani et al., 1987), our results are the first to suggest a possible mechanism of this effect. It is still unclear, however, how DEX modulates BBB function, though it has been suggested that it might inhibit mediators such as prostaglandins (Hoeck et al., 1993), tumor necrosis factor-α (Steer et al., 2000) and/or nitric oxide (Radomski et al., 1990), each of which has been reported to influence BBB permeability (Boje, 1996; Didier et al., 2003; Sharief and Thompson, 1992; Zuckerman et al., 1994). Clearly, more work needs to be done in this area.

In conclusion, our data showed that DEX was effective in preventing brain demyelination that was caused by the rapid correction of hyponatremia in a rat model of CPM, possibly by inhibiting BBB disruption. Early treatment with DEX, which is already commonly used in the clinical setting, during the correction of hyponatremia could be useful in preventing CPM.

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