Vacuolar Pathology in the Median Eminence of the Hypothalamus Following Hyponatremia

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Abstract

The median eminence of the hypothalamus is an important conduit by which neurosecretory hormones from hypothalamic nuclei are delivered to the pars nervosa (neural lobe) of the pituitary en route to the bloodstream. Dilutional hyponatremia was produced in adult rats to determine the effect on the morphology of the median eminence of the hypothalamus. Hyponatremia was caused by reducing electrolyte and organic osmolyte reserves in order to block the excretion of water through delivery of the nephrotoxin mercuric chloride (HgCl2). Histological examination of the brain 1 day following a hyponatremic insult revealed vacuolation within the median eminence of the hypothalamus. No other lesions were found in other parts of the brain following hyponatremia. The hyponatremic lesion consisted of a band of closely packed vacuoles that crossed the floor of the third ventricle. Vacuoles associated with hyponatremia were predominantly in the subependymal, fiber, reticular, and palisade layers of the median eminence. Vacuolation was not observed in the tanycyte layer of the median eminence. This study indicates that the median eminence is a potentially vulnerable site in human hyponatremic conditions that should be evaluated further in relevant animal models.

Keywords

Adenohypophysis; Cisterns; Hyponatremia; Pituitary; Tanycyte; Vacuoles

INTRODUCTION

Hyponatremia, also referred to as ‘water intoxication,’ is the result of the progressive hypotonicity of body fluids (1,2). Severe hyponatremic insults can result in brain edema and death. Hyponatremia is a common complication of disorders in which there is rapid sodium loss (e.g. profuse diarrhea or vomiting), or when excess water accumulates at a higher rate than can be excreted (3). Hyponatremia is also observed following the aberrant secretion of the antidiuretic hormone vasopressin; antagonists of vasopressin are effective for the...
treatment of hyponatremia under certain conditions (4-6). Vasopressin is a secretory product of the magnocellular supraoptic and paraventricular nuclei in the hypothalamus and passes through the median eminence en route to the pars nervosa (neural lobe) of the pituitary gland where it is discharged into the bloodstream (7,8). Hyponatremia is also observed when serum sodium is rapidly diluted (e.g. hypertriglyceridemic and hyperglycemic conditions and in patients with various illnesses when excessive quantities of hypotonic fluids are administered for therapy [9]), and it is associated with a fatal syndrome of central diabetes mellitus and diabetes insipidus (10). Importantly, hyponatremia is the most common electrolyte balance disorder found in hospitalized patients (11,12). Hyponatremia can be associated with neuropsychiatric disorders, including schizophrenia, whereby affected individuals voluntarily consume excessive quantities of water (polydipsia) (5,13).

In rats, acute hyponatremia has also been demonstrated to be a secondary insult following traumatic brain injury in which it may involve the swelling of astrocytic end feet near perivascular spaces and the upregulation of the glial water channel aquaporin-4 (14,15). Renal tubular necrosis with subsequent pituitary damage can be produced in relevant animal models by delivery of a nephrotoxin such as intravenous injection of HgCl$_2$ or intraperitoneal injection of D-serine (16). Our group has reported hydropic degeneration of the pituitary pars distalis (adenohypophysis) in both of these experimental rat models of uremia, which are associated with hyponatremia (16,17). Specifically, we demonstrated that delivery of HgCl$_2$ (as well as injection of nephrotoxic levels of the amino acid D-serine) caused hyponatremic insults within the adenohypophysis that were indistinguishable morphologically from one another (17). Despite the clinical importance of understanding brain and pituitary changes during hyponatremic insults, little morphologic or mechanistic information is currently available in relevant animal models. The present study aimed to determine whether an acute hyponatremic insult in rats could produce brain as well as pituitary pathology.

**MATERIALS AND METHODS**

All procedures were approved by the Institutional Animal Care and Use Committee of the Nathan Kline Institute. Lewis rats of either sex (n = 19) were kept in hanging shoebox type plastic cages with corncob bedding. Laboratory Rodent Diet 5001 chow (Purina, Scott’s Distribution, Hudson, NH) was available ad libitum. Rats were used when 7 to 10 weeks old (180-260 g). At the time of experimentation, rat chow was replaced by pure sucrose cubes (Domino Dots, West Palm Beach, FL). Metal grids were placed at the bottom of each cage to reduce coprophagy. Rats were given an i.v. injection of HgCl$_2$ (Sigma, St. Louis, MO) in 1 mg/ml distilled water while anesthetized with isoflurane, as previously described (17). There were 2 dosing regimens, i.e. 0.25 ml/100 g body weight (n = 9) and 0.5 ml/100 g body weight (n = 5). Distilled water (10 mg/100 g body weight) was injected s.c. 10 minutes after the HgCl$_2$ injection on the left side of the body; this was repeated 5 hours later on the right side of the body. To prevent leakage, the 20-gauge needle was advanced alternately through the subcutis and then the dermis 3 or 4 times before the water was injected into the subcutis (valve technique). Control rats (n = 5), were treated identically except they received distilled water instead of HgCl$_2$.

Rats were killed by exsanguination while under CO$_2$ anesthesia. Serum sodium levels were assessed by preparing blood taken from the inferior vena cava at the time of death. Sodium was assayed by atomic absorption spectrophotometry, as described (18,19). The brains were removed quickly, placed into Bouin’s fluid and the base of the skull was immersed in Bouin’s fluid to fix the pituitary in situ for 24 hours at 22°C. The brain and pituitary were embedded in paraffin, serially sectioned at 5 μm, and selected sections stained with hematoxylin and eosin (Sigma) and with periodic acid-Schiff-hematoxylin (PAS) (18,19).
Particular emphasis was placed on evaluating the median eminence based upon previous results (16,17). The median eminence measures approximately 1.6 to 1.8 mm in the anterior-posterior plane in fixed rat brain (20). Tissue sections were evaluated at 4 levels throughout the extent of the median eminence to assess the ependymal (tanycyte) layer, subependymal layer, fiber layer, reticular layer, and palisade layer, as well as the pars tuberalis of the infundibulum (19). Tissue sections were examined at 400x magnification and vacuoles throughout the median eminence were quantified as previously described (16,17). Vacuolar pathology was assessed on a semiquantitative scale as follows: 0, no vacuolar pathology; 1+, scattered vacuoles in any one of the layers; 2+, numerous vacuoles in 1-2 layers, sometimes grouped together; 3+, profuse vacuolation, organized in short bands across part of the width of the median eminence and spanning at least 2 laminae; 4+, severe vacuolar pathology organized in long bands, spanning the median eminence. This approach obviated concerns regarding tissue selection bias (17-19). Vacuoles in the median eminence had no detectable content and were interpreted as a hydropic change (16,17).

RESULTS

Sodium levels were significantly decreased in hyponatremic rats (110.43 ± 7.59 mEq/L) vs. control rats (145.4 ± 1.67 mEq/L) (t-test; p < 0.001). No differences in serum sodium levels were observed between the 2 dosing conditions of HgCl$_2$ (low, 111.78 ± 9.07 mEq/L; high, 108.0 ± 3.39 mEq/L). Vacuolar pathology was present in the median eminence of 14/15 (93.3%) of rats with acute dilutional hyponatremia (Table). Control rats without hyponatremia did not display vacuoles in the median eminence. No pathological alterations were observed in the arcuate, supraoptic, or paraventricular nuclei of the hypothalamus or other cortical or subcortical areas. Rats with hyponatremic insults also had hydropic degeneration of the anterior pituitary, as described (17). The 2 doses of HgCl$_2$ caused nearly similar semiquantitative scores of vacuolar pathology (Table), although the lower dose had 2 rats with milder (1+) pathology. In rats with hyponatremia, a band of closely packed vacuoles extended across most or all of the median eminence, encompassing the fiber and reticular layers (Figs. 1-3). The ependymal layer (containing the tanycytes) did not display significant vacuolar pathology, although vacuoles filled the adjacent subependymal layer as well as the fiber layer and reticular layer (Fig. 1). Vacuolated bands were also observed consistently within the palisade layer (Figs. 1, 2). Thus, nearly the full anterior-posterior extent of the median eminence was subject to vacuolar pathology, and laterally the vacuoles reached the level of the tuberoinfundibular sulcus, which effectively demarcated the lateral edge of the median eminence. Vacuoles were usually elliptical in shape with the long axis oriented in the dorsoventral plane, similar to the tanyctye processes and the fibers of the palisade layer. Glial nuclei were visualized in and around the band of vacuoles, but the vast majority of the vacuoles were optically empty. Vacuolar pathology also appeared optically empty in the PAS stain, indicating that these vacuoles did not contain glycogen.

Immediately lateral to the median eminence and the floor of the third ventricle, the adjoining hypothalamic tissue often contained clusters of a few cisternal-like structures of a different morphological appearance than the aforementioned vacuoles. Unlike the vacuoles caused by hyponatremia, the cisternal-like structures were readily observed in both control and experimental rats. These round elements, termed “hypothalamic cisternae” (19,21-23), were present in the lateral margins of the median eminence and the tuberohypophysial sulci and were markedly different in location and shape from the vacuoles of the median eminence associated with the hyponatremic insult (Fig. 1-3). Importantly, they were seen in rats with or without vacuolar degeneration of the median eminence (Fig. 1).
DISCUSSION

Vacuolar pathology within the median eminence appeared to be specific to the hyponatremic insult; no similar vacuoles have been reported in the median eminence (or other brain regions) of rodents using a variety of experimental paradigms including excitotoxicity, hyperlipemia, inflammation, lithium treatment, gonadal steroid delivery, and vitamin D treatment, among others (18,19,24-29). Dilutional hyponatremia was generated in the present study through injection of the nephrotoxic agent HgCl$_2$ similar to our previous studies (16,17). The nephrotoxicity of HgCl$_2$ delivery is well established (30,31), notably within the dosing range used in this report (16,17). Mercury, particularly in the vapor form, is also neurotoxic (32). Mercuric salts including HgCl$_2$, although neurotoxic to varying degrees, appear to be less neurotoxic than the vapor form and are less blood-brain barrier penetrant (32). Glial cells, including astrocytes, may be potential mediators of HgCl$_2$ toxicity within the brain, although their contribution to hypothalamic and/or pituitary changes remain unknown (33,34). To our knowledge, the potential of direct neurotoxicity of intravenous HgCl$_2$ delivery on the mediobasal hypothalamus and pituitary has not been determined. Accordingly, we cannot formally exclude the possibility that HgCl$_2$ has some direct effects upon the median eminence. However, this is unlikely within the current paradigm, as the 2 doses of HgCl$_2$ produced equivalent levels of serum sodium depletion as well as vacuolar pathology within the median eminence, consistent with our previous analysis (17).

The present objective was to determine whether acute dilutional hyponatremia could produce lesions in brain and pituitary as observed in humans with a fatal syndrome of diabetes mellitus and diabetes insipidus (10). The vacuolar, fluid-filled character of the lesions could have been expected inasmuch as hyponatremia and water intoxication are almost synonymous. However, the location of these vacuoles within the median eminence of the hypothalamus was not anticipated. There is experimental evidence that cerebrospinal fluid can pass directly from the third ventricle through the median eminence (35-37). Moreover, orientation of the elliptical vacuoles in hyponatremic rats is in register with the orientation of the tanycytes and their processes. Therefore, the content of the vacuoles observed in the median eminence following hyponatremia may be cerebrospinal fluid. To our knowledge, vacuolar pathology of this type following a hyponatremic insult has not been reported in the brain. It has been reported that hyponatremic rats exhibit degeneration of the adenohypophysis (17) as well as the vacuolar pathology within the median eminence. Moreover, the present findings within the median eminence are similar to peripheral vacuolar changes in renal glomeruli following nephrotoxic insults (38,39).

The layers of the median eminence from dorsal (upper) to ventral (lower) are the ependymal layer (tanycyte layer), subependymal layer, fiber layer, reticular layer, and palisade layer (19,20). The palisade layer is readily identified by the closely compacted parallel fibers perpendicular to the pars tuberalis of the pituitary and by the highly vascularized (portal circulation) lowest portion of the layer (19,20). The delicate arachnoid membrane is attached to the pars tuberalis. The observation that vacuolar pathology following an acute hyponatremic insult was selective for the laminae of the median eminence is intriguing from several perspectives. A goal is to determine the intrinsic morphological, molecular, and or cellular underpinnings that cause this selective vulnerability within the median eminence. The relative sparing of vacuolar pathology within the tanycyte layer is also of interest, as this layer displays more mitotic figures (likely originating from specialized astrocytes called pituicytes) following osmotic stress than the other median eminence laminae (19). Further, we suspect that the cisterns that are normally present within the median eminence predispose this region to vacuolation. Tangential evidence for this hypothesis is supported by a study whereby adrenalectomy in adult rats increased the abundance of hypothalamic cisterns (40).

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Since adrenalectomy causes low serum sodium, this observation is consistent with our findings in the present hyponatremic model. Additional ultrastructural assessment within the laminae of the median eminence is warranted following hyponatremia to determine the precise morphological characteristics of these vacuolar lesions. Furthermore, assessment of vasopressin levels within the hypothalamus and the median eminence in hyponatremic models may prove useful to determine the potential mechanism of action in human hyponatremic syndromes. Although numerous models and systems exist to study the effects of hyponatremia peripherally, relatively little is available for the brain and pituitary. Our morphological results in a rat model of acute hyponatremia suggest that the median eminence is a crucial structure to monitor and assess for understanding mechanisms underlying the hyponatremic process as well as providing a substrate (vacuolar pathology) to test interventions aimed at reducing the negative sequela associated with hyponatremia.

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REFERENCES


Figure 1.
Low- and high-magnification images of the mediobasal hypothalamus in coronal sections from a control (A) and hyponatremic (B-D) rats. The median eminence extends below the third ventricle (III v.), across the bottom of the photomicrographs. Cisterns (arrows) located just lateral to the median eminence often appeared to be attached to the pars tuberalis at the level where it separates the median eminence from the rest of the hypothalamus. (A) Control rat. (B) Vacuolation is symmetrically distributed in the subependymal layer of the median eminence of a hyponatremic rat (scored at 1+). Tanyocytes, which form the floor of the third ventricle, are not vacuolated. (C) Vacuolation is distributed symmetrically in the subependymal, fiber, and reticular layers (scored at 3+). (D) Vacuoles are prominent in the fiber, reticular and palisade layers of the median eminence (scored at 4+). Vacuoles on the left side palisade layer adjacent to the pars tuberalis are confluent with an asymmetric group of larger cisterns. Hematoxylin and eosin. Scale bars: A-D = 20 μm.
Figure 2.
Low (left panels) and higher (right panels) photomicrographs showing vacuolation in the fiber, reticular, and palisade layers following a hyponatremic insult. (A) Clusters of vacuoles are concentrated in the palisade layer adjacent to pars tuberalis of pituitary (scored at 3+). Numerous cisterns are also seen at higher magnification. (B) Widespread, symmetrical vacuolar pathology throughout the median eminence (scored at 4+). In this rat, hypothalamic cisterns were scarce and separated laterally from vacuoles within the median eminence. Scale bar = 20 μm.
Figure 3.
Prominent vacuolation within the median eminence following hyponatremia. Low (left panel) and higher (right panel) photomicrographs illustrating profuse vacuoles throughout the median eminence but not the adjacent mediobasal hypothalamus. (A) Severe vacuolar lesions within the median eminence of a hyponatremic rat (scored at 4+). The shallow tuberoinfundibular sulcus (arrow) is seen near cisterns within the lateral margins of the median eminence. (B) Vacuolation throughout the subependymal, fiber, reticular, and palisade layers of the median eminence. Hypothalamic cisterns are large and numerous in the lateral margins of the median eminence. Scale bars: A, B = 20 μm.
Table 1

Semiquantitative Scoring of Vacuolar Pathology within the Median Eminence and Serum Sodium Levels in Individual Rats following Treatment with HgCl₂

<table>
<thead>
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