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Morphological changes in the brain during experimental hyponatraemia. Do vasopressin and gender matter?

Ewa Koźniewska[1,3], Roman Gadamski[2], Katarzyna Klapczyńska[1], Renata Wojda[2], Janina Rafałowska[2]

[1]Laboratory of Experimental Neurosurgery, Department of Neurosurgery and [2]Department of Experimental and Clinical Neuropathology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw; [3]Department of Experimental and Clinical Physiology, Medical University of Warsaw, Warsaw, Poland

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Abstract

Hyponatraemia is the most common electrolyte balance disorder occurring in hospitalized patients. The disease results frequently from inappropriate secretion of vasopressin (SIADH). It has been evidenced that the brain consequences of hyponatraemia are more dramatic in young females than in men or postmenopausal women. Since both vasopressin and oestrogen have been reported to inhibit ion fluxes essential for the adaptation of the brain to the lowering of serum sodium concentration, we sought to study the effect of acute and chronic hyponatraemia or hyponatraemia associated with vasopressin on brain morphology in male and female rats. Hyponatraemia was induced with vasopressin (AVP) or with desmopressin (dDAVP) in 12 male and 12 female adult Wistar rats for either 3 hours (acute) or 3.5 days (chronic). The brains of the animals with diagnosed hyponatraemia were fixed in 10% formalin and, following the standard procedure, stained with haematoxylin and eosin. Acute hyponatraemia resulted in white matter oedema with no obvious differences between genders or between groups with AVP- or dDAVP-induced hyponatraemia. Although in chronic hyponatraemia most neurons and astrocytic nuclei appeared to be normal, some neurons were swollen or ischaemic ("dark" neurons) and astrocytes showed a weak reaction. The most spectacular differences between males and females were found in the appearance of blood vessels. Swollen endothelial cells were observed more frequently in female than in male brains and in AVP- than in dDAVP-induced hyponatraemia. The widened Virchow-Robin spaces indicated perivascular oedema and blood-brain barrier damage. The results point to limited vascular adaptation to AVP-associated hyponatraemia in female gender.

Key words: *hyponatraemia, hypo-osmotic brain swelling, vasopressin, sex hormones*

Introduction

Hyponatraemia is a common electrolyte disturbance observed in hospitalized patients. It is defined as a decrease in the serum sodium concentration below 136 mmol per litre [2]. The most common form of hyponatraemia is a dilutional one. It is caused by water retention due to inappropriate secretion of antidiuretic hormone (SIADH) and is associated with hypo-osmolality and hypotonicity [2].

Hyponatraemia is particularly dangerous when occurring in patients after brain trauma or subarachnoid haemorrhage since it worsens their outcome and increases the risk of permanent brain damage or

Communicating author:

Ewa Koźniewska, PhD, Laboratory of Experimental Neurosurgery, M. Mossakowski Medical Research Centre of Polish Academy of Sciences, 5 Pawińskiego St., 02-106 Warsaw, Poland; tel. +48 22 608 64 89, Email:ewak@cmdik.pan.pl

death in these patients [8,10,16]. The main adverse factor in hyponatraemia is hypotonicity. It creates an osmotic gradient across the cell membrane leading to the shift of water from the extracellular to the intracellular compartment, which results in brain swelling or aggravates the pre-existing one [21,37].

Neurological symptoms associated with hyponatraemia depend on the rate of the decrease of serum Na^+ concentration [35,37]. When it decreases slowly (>48 hours, chronic hyponatraemia) brain cells adapt to hypo-osmolality by extruding ions and inorganic osmolytes which restores their volume to near normal [2,37]. Brain symptoms of chronic mild hyponatraemia (serum sodium concentration >120 mmol/l) are subtle and non-specific, e.g. headache, lethargy and nausea. When serum sodium concentration decreases rapidly (<48 hours, acute hyponatraemia) and below 120 mmol/l brain cells cannot fully adapt, which is manifested by symptoms of brain oedema. This may result in irreversible neurological damage, respiratory arrest, brain herniation, and even death [17,37].

Although the incidence of hyponatraemia is similar among men and women, brain damage was reported to occur mainly in young (menstruant) females and prepubertal individuals of either gender [4-6]. The greater risk of brain injury in premenopausal women seems to be associated with the inhibitory effect of the female sex hormones on brain adaptation to osmotic swelling [14,15].

Vasopressin, the most frequent cause of hyponatraemia, has also been reported to limit brain adaptation to osmotic swelling, to aggravate ischaemic brain oedema and to impair blood-brain barrier functions [19,29,40]. In accordance with these experimental studies are clinical data on the adverse effects of vasopressin in patients with hyponatraemia [1,9].

The aim of the present study was to compare the morphology of the brain during acute and chronic experimental hyponatraemia and to examine the impact of vasopressin and female gender on both types of hyponatraemia.

Material and Methods

The experimental protocol was approved by the Local Ethical Committee.

Fourteen male and fourteen female adult Wistar rats (260 to 300g) were used for this study. Hyponatraemia was induced in 24 rats, and 4 rats served as a normonatraemic control group. In 12 rats acute hyponatraemia was induced for 3 hours by subcutaneous injection of vasopressin (AVP, 1 µg) or its kidney-affecting analogue desmopressin (dDAVP, 0.4 µg) in conjunction with intraperitoneal administration of 140 mM glucose/water solution in a dose of 9-12% body weight. In the remaining 12 rats chronic hyponatraemia was induced over 3.5 days prior to the experiment by the subcutaneous twice-daily administration of 1 µg AVP or 0.4 µg dDAVP along with intraperitoneal 8% body weight of 140 mM glucose/water solution.

On the day of the experiment, 3 hours after the last administration of AVP/dDAVP/140 mM glucose the animals were anaesthetized with i.p. chloral hydrate and decapitated.

Their brains were fixed in 10% formaldehyde for at least 96 hours, dehydrated, embedded in paraffin and cut into 8 µm thick sections. The sections were stained using standard histological methods: haematoxylin-eosin (HE) and immunostaining with antibodies against glial fibrillary acidic protein (GFAP). Trunk blood was collected for the measurement of plasma Na^+ concentration using flame photometry.

The following reagents and drugs were used: vasopressin (Sigma-Aldrich, Poland), desmopressin (Ferring, Poland), GFAP (Dako, Denmark). Vasopressin was prepared daily from aliquots of 1 mM stock solution stored at -20°C.

Statistical analysis of plasma sodium concentration was made using analysis of variance (ANOVA) with post-hoc Tukey test. Probability of $p<0.05$ was considered statistically significant. The data are presented as means ± SE.

Results

Acute hyponatraemia

Concentration of Na^+ in the plasma of male rats 3 hours after induction of acute hyponatraemia was 107±3 mmol/l in AVP-induced and 117±2 mmol/l in dDAVP-induced hyponatraemia ($p<0.02$, Student's t test). In females plasma concentration of Na+ at the same time point was 111±2 and 118±2 mmol/l in AVP- and dDAVP-induced hyponatraemia, respectively. There were no differences between both female groups. There were also no differences between males and females and AVP- or dDAVP-induced hyponatraemia as analyzed with one way ANOVA and post-hoc Tukey test. Hyponatraemic Na^+ plasma levels were, however, significantly lower than normonatraemic

ones (142±1 mmol/l, pooled from male and female rats) in all rats ($p<0.001$).

The main finding of this series of studies was widespread white matter oedema (Fig. 1) with no obvious differences between genders or between groups with AVP- or dDAVP-induced hyponatraemia.

Marked vacuolar degeneration of choroid plexuses was also noted (Fig. 2).

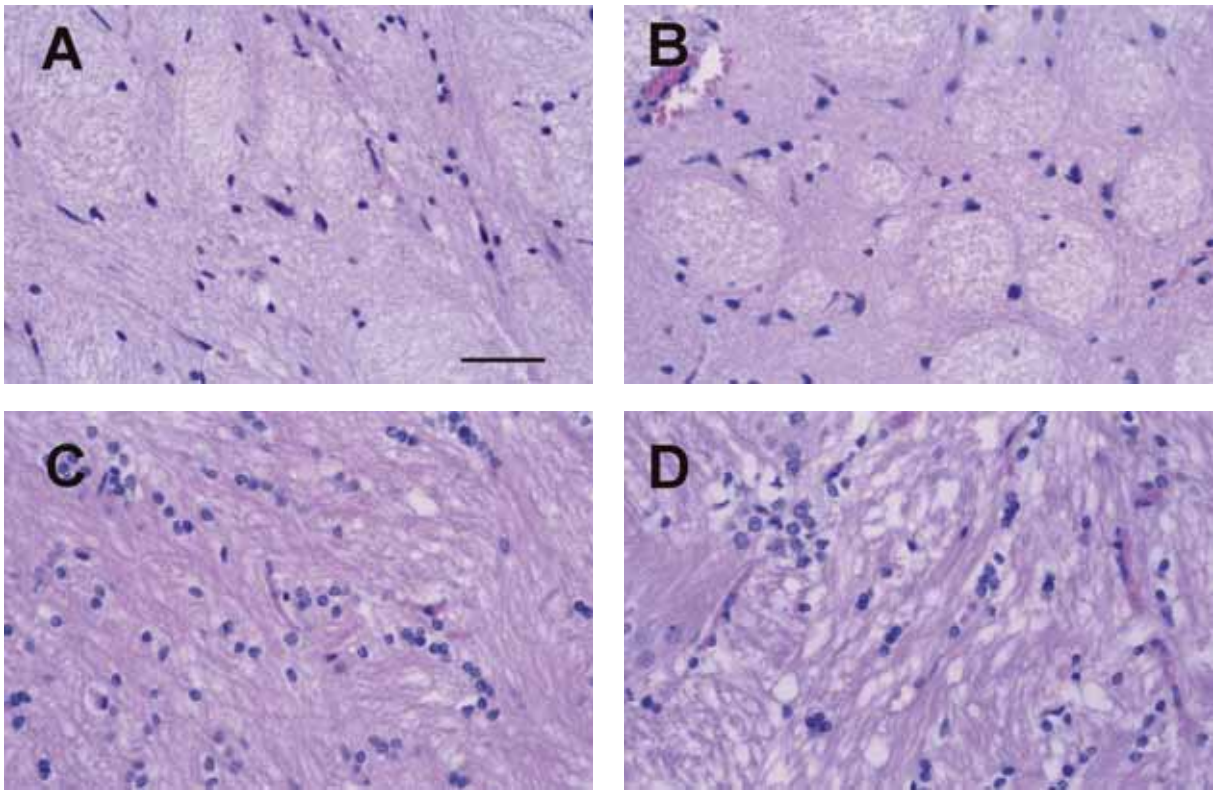

Fig. 1. HE stained white matter sections of the brain submitted to 3 hours hyponatraemia. A and C – male rat with AVP- or dDAVP-induced hyponatraemia, respectively; B and D – female rat with AVP- or dDAVP-induced hyponatraemia, respectively. The bar indicates 50 µm

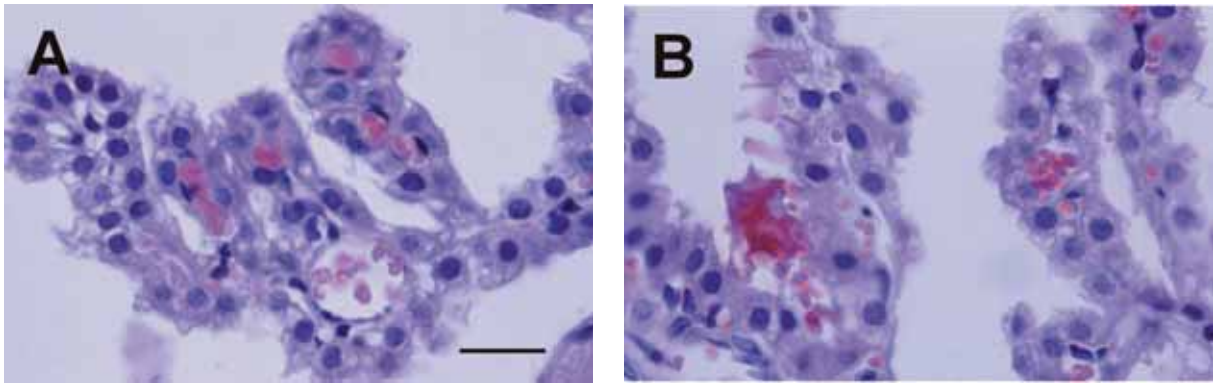

Fig. 2. HE stained sections of choroid plexus of the lateral ventricle of the female brain with acute hyponatraemia. A – hyponatraemia induced with AVP; B – hyponatraemia induced with dDAVP. The bar indicates 100 µm

Chronic hyponatraemia

Plasma sodium concentration after 3.5 days of hyponatraemia did not differ between female and male rats irrespective of the method of its induction (AVP vs. dDAVP). In male rats it was 112±2 mmol/l in AVP-induced and 110±5 mmol/l in dDAVP-induced hyponatraemia. In females plasma concentration of Na+ at the same time point was 115±1 and 102±5 mmol/l in AVP- and dDAVP-induced hyponatraemia, respectively.

Analysis of brain morphology after 3.5 days of hyponatraemia revealed differences both between AVP- and dDAVP-induced hyponatraemia as well as between male and female. Although most of the neurons and astrocytic nuclei in the cerebral cortex (Fig. 3), hippocampus and other subcortical structures appeared to be normal, some of the neurons were swollen or ischaemic ("dark" neurons) (Fig. 3 B, D) and astrocytic nuclei were shrunken (Fig. 4). These changes were slightly more pronounced in females and in AVP-induced hyponatraemia (Fig. 3 B, D). The most spectacular differences between males and females were found in the appearance of blood vessels. Swollen endothelial cells (Fig. 3 B, D), subependymal exudation of cerebrospinal fluid and weak astrocytic reaction were obvious in female brains (Fig. 4). The widened Virchow-Robin spaces indicated perivascular oedema and blood-brain barrier damage. These changes were seen in male rats only occasionally (Fig. 3 A, C). They were, however, less dramatic in dDAVP-induced hyponatraemia.

Discussion

This study addressed the issue of brain adaptation to hyponatraemia from the perspective of morphological changes in the brain. This is a different approach than in other studies performed earlier in which adaptation to hyponatraemia was assessed by measuring the amount of brain water [27,41,42]. The main finding of our study is that during acute hyponatraemia brain cells and white matter swell to

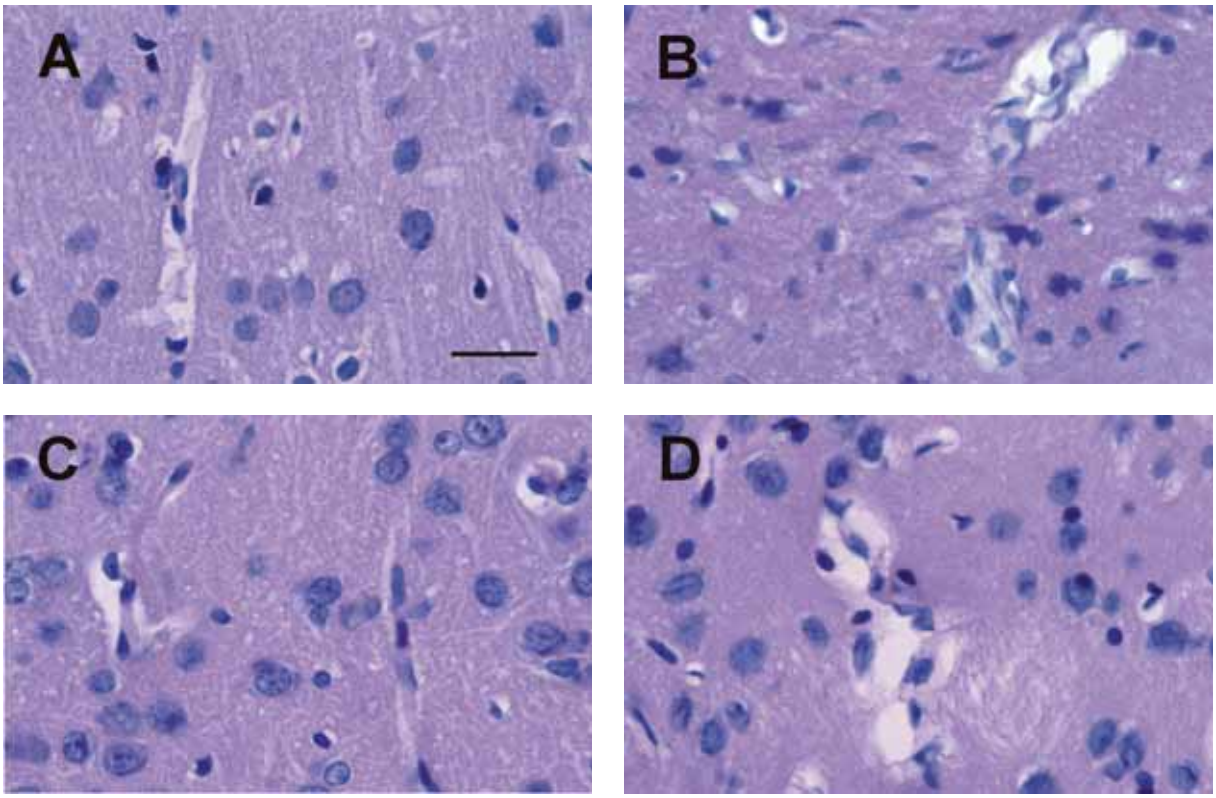

Fig. 3. HE stained cortical sections of the brain submitted to 3.5-day hyponatraemia. A and C – male rat with AVP- or dDAVP-induced hyponatraemia, respectively; B and D – female rat with AVP- or dDAVP-induced hyponatraemia, respectively. The bar indicates 100 µm

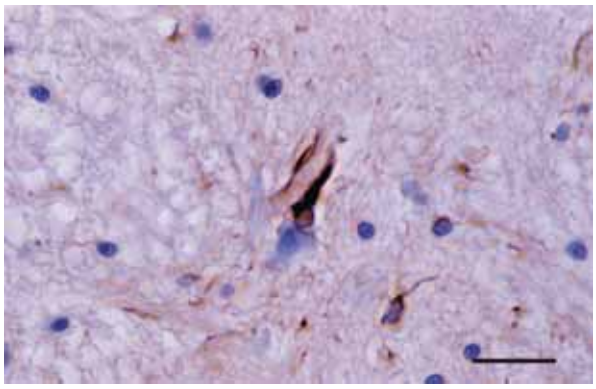

Fig. 4. Astrocytic degenerative changes within white matter of the brain stained with GFAP antibody. The bar indicates 100 µm

a similar extent irrespective of the gender and presence of vasopressin. Interestingly, during chronic hyponatraemia, vasopressin and oestrogen seem to hinder the process of adaptation to hypo-osmolality. The worst adaptation to hyponatraemia lasting 3.5 days in our study was observed in females with AVP-induced hyponatraemia and was most pronounced in blood vessels. According to our knowledge based on the available literature there are no data on the morphology of the brain subjected to hyponatraemia or, particularly, on the comparison between AVP- and dDAVP-induced one.

Morphology during acute hyponatraemia in our study matches the common view of the cellular response to hypotonicity. Hypotonic hyponatraemia results in immediate cell swelling due to the osmotically driven flow of water into the intracellular space [21,34,37,41]. There is no difference in the amount of brain water at this acute stage between male and female or AP- and dDAVP-induced hyponatraemia in our study (data not shown).

The response of the cells to osmotic swelling is a loss of intracellular solutes in order to re-establish the baseline size [21,37]. This response, known as regulatory volume decrease (RVD), is observed in a variety of cells, although it occurs with different rates. It may last from 30 min to more than 24 hours [18].

It has been established that RVD depends on the extrusion of intracellular solutes such as Na^+, K^+ and Cl^- ions into the extracellular space, thus resulting in a decrease of cytosolic osmolality and amount of water [12,18,27,28]. During longer lasting, chronic hyponatraemia (more than 48 hours) elimination of other intracellular constituents such as low molecular weight organic solutes (osmolytes) participates in RVD [22,25,36]. Decrease of the brain content of taurine, glutamine and inositol after 2 days of dDAVP-induced chronic hyponatraemia ranged from 60 to 84%, which was nearly 3 times larger than the fall in brain electrolytes [43]. According to Lien et al. [25], after 3 days of hyponatraemia the decline of electrolytes accounts for 72% of the decrease of brain osmolality, whereas the remaining 23% depends on the elimination of organic osmolytes. Extrusion of osmolytes is considered as a defence not only against accumulation of water but also as a homeostatic mechanism against the dramatic changes in intracellular electrolyte concentrations. Some authors postulate also participation of selected organic osmolytes in the early phase of RVD [36].

Our results suggest that adaptation to chronic hyponatraemia is affected by both vasopressin and gender, at least as far as the morphological appearance of the brain tissue is concerned. The swollen endothelia indicate impaired ability of these cells to extrude water, which is particularly striking in vasopressin-induced hyponatraemia.

The widened Virchow-Robin spaces suggest vasogenic oedema, which may result from the increased permeability of brain microvessels to water.

The difficulty of endothelial cells to adapt in the presence of vasopressin is supported by the data published by others that vasopressin impairs ionic mechanisms responsible for the regulation of cell volume under conditions of hyponatraemia [19,29,33]. According to O'Donnell co-transport of Na^+-K^+-Cl^- is essential for the regulation of cell volume in endothelial cells [28]. In her studies co-transport of Na^+-K^+-Cl was inhibited in cultured bovine aortic endothelial cells exposed to hypotonic solution and this response correlated with the decrease in cell volume. Recent studies by O'Donnell et al. demonstrate that AVP stimulates Na^+-K^+-Cl co-transport in cerebral microvascular endothelial cells via the V_1 receptor [29]. Although the conclusion of the latter study was that AVP participates in ischaemia-induced oedema formation through the stimulation of the above transporter, in the context of our study O'Donnell's results speak in favour of inhibition of the RVD response. Vasopressin has also been reported to impair regulatory volume decrease by inhibiting calcium-coupled sodium efflux [19]. These effects were V1a receptor-dependent.

In the context of our results it is important that V_{1a} receptors are expressed in the rat brain not only

in neurons but also in astrocytes and intraparenchymal blood vessels [31,38,39]. According to Szmydynger-Chodobsla et al. [38] strong V_{1a} receptor immunoreactivity is observed in astrocytic processes associated with intraparenchymal blood vessels. In contrast, V_2 receptor mRNA in the adult rat brain was found only in the cerebellum [20].

Another factor known to be important for the homeostasis of cell volume, Na^+/K^+ ATPase, has been shown to be inhibited by oestrogens [14,15]. It should be stressed that oestrogen is considered as one of the most important cardiovascular protective hormones under physiological conditions, and has beneficial effects on blood flow, resulting in vasodilatation due to the stimulation of the release of NO and vasodilatory prostaglandins from the endothelium [11,30]. Many experimental studies have also demonstrated a protective effect of oestrogen against ischaemic brain damage [26,44,45]. There are, however, some controversies concerning oestrogen-induced neuroprotection [7,13,32]. According to Santizo et al. [32] this protection may be lost in the case of ischaemia associated with another disease. In their experimental studies performed in diabetic female rats subjected to ischaemia, oestrogen pretreatment was associated with a much greater cell loss and neurological impairment compared to intact females. Thus, under their experimental conditions oestrogen was not beneficial. Our results suggest that oestrogen may impair the defence against osmotic cell swelling. In this context it is worth mentioning our previously published study on the impairment of cerebral microvascular regulation in rats with chronic AVP-induced hyponatraemia [23,24].

Taken together, our study suggests that vasopressin in conjunction with oestrogen limits vascular adaptation to hyponatraemia. The exact mechanism of this interaction is at present unclear and therefore requires further studies.

Our results are partially and indirectly supported by clinical observations that aquaretics with V_{1a}/V_2 receptor antagonism (i.e., vascular/kidney antagonism) are very promising in the treatment of patients with hyponatraemia [2,16].

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