ORIGINAL ARTICLE

Neuronal excitability after water intoxication in young rats

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Submitted: 2014-07-03 Accepted: 2014-07-06 Published online: 2014-07-15

Key words: **water intoxication; brain water content; neuronal excitability; AQP 4; ontogeny**

Neuroendocrinol Lett 2014; **35**(4):274–279 **PMID:** 25038600 NEL350414A04

Abstract

OBJECTIVES: Our previous experiments with animal models revealed that water intoxication induces brain oedema and opens plasma membranes. Present study is aimed to determine whether the standard method of hyperhydration can influence cerebral microenvironment also in young rats. Neuronal functions were tested by standard electrical cortical stimulation.

METHODS: Hyperhydration was induced by administration of distilled water (DW) intraperitoneally. Three groups of young rats were used: 12, 25, and 35-day-old. Cortical excitability was tested 19 to 20 hours after DW administration by electrical stimulation of the sensorimotor cortex with intensity necessary to elicit cortical afterdischarges (AD). Water content in the brain was estimated by dry/wet ratio and value of natremia by standard biochemical examination. Control animals of the same age groups were tested in the same way, only they did not receive DW.

RESULTS: Brain water content in hyperhydrated animals was smaller than in controls in all studied age groups. Natremia was the same (normal) in both the hyperhydrated and control animals aged 25 days. Excitability of cortical neurons in young hyperhydrated animals was significantly inhibited in comparison to the same age groups of controls.

CONCLUSION: Hyperhydration induced in young rats (12, 25, 35-day-old) had different effects than in adults. Absence of hyponatremia, lower water content in the brain and significant inhibition of cortical excitability can be explained on the basis of ontogenetically dependent aquaporine expression (AQP 4) and different activity of ionic membrane transporters.

Abbreviations:

DW	- distilled water
AD	- afterdischarges
AQP	- aquaporin
CSWs	- cerebral salt wasting syndrome
SIADH	- syndrome of inappropriate antidiuretic hormone secretion
ADH	- vasopressin
CNS	- central nervous system
SMF	- static magnetic field

INTRODUCTION

One of the essential conditions which guarantee integrity of the internal environment of the nervous tissue is the water and ionic homeostasis (Pokorný *et al.* 2002; Kozler & Pokorný 2012). Its maintenance or disruption depends also on the water and ionic balances between the intra- and extracellular compartment of the brain. The flow of water and ions is controlled by a complex mechanism of the cell membrane permeability, namely by the control of ionic channels and aquaporins. Perturbations of this balance usually result in the alterations of brain functions (Agre *et al.* 2004). Changes in the water and ionic distribution can be rapid and fatal as it is in the diffuse brain injury (Giza *et al.* 2001) and cerebral ischemia (Dirnagel *et al.* 1999). The disbalance can also develop slowly, e.g. in hyponatremic syndromes of cerebral salt wasting syndrome (CSWs) or in syndrome of inappropriate antidiuretic hormone secretion (SIADH) (Peters 1950; Schwartz 1957).

The role of brain microenvironment in the function of neuronal circuits can be experimentally studied e.g. on the basis of their ability to respond to a stimulus. Above threshold electrical stimulation of cerebral cortex can induce synchronized activity of neurons which manifests in cortical afterdischarges (AD) accompanied with specific motor patterns (head and extremity jerks, whole body twitches, loss of the righting reflex) (Racine 1972), and manifesting in EEG by the shape and duration of graphoelements (Schwartzkroin *et al.* 1998). The pattern of stimulation response in cortical neurons and the degree of seizure susceptibility is also age dependent (Swan *et al.* 1993; Seifert *et al.* 2010).

Water flow in the brain microenvironment is controlled by aquaporins (namely AQP 4) and it develops during the ontogeny (Li *et al.* 2013). In our earlier experiments we found that in adult rats alteration of brain microenvironment induced by hyperhydration results in the increased permeability of cell membranes accompanied with penetration of high-molecular substances into the brain (Kozler & Pokorny 2003) with elevation of the water content in the brain (Kozler *et al.* 2013).

The aim of the present study was to find whether hyperhydration in young animals can influence excitability of cortical neurons.

MATERIAL AND METHODS

All experiments were approved by the Ethical Committee of the First Faculty of Medicine (Charles University in Prague) and were in agreement with the Guidelines of the Animal Protection Law of the Czech Republic and Guidelines for the treatment of laboratory animals EU Guidelines 86/609/EEC.

1. Electrophysiology

In experiments male Wistar albino rats of our own bred aged 12, 25, and 35 days were used. Cortical excitability was tested by electrical stimulation of the right sensorimotor cortex. Electrocortical activity (ECoG) was recorded from the left sensorimotor and both occipital cortical regions. Parameters of stimulation were set at: rectangular bipolar pulses with duration 0.5 ms, intensity necessary for eliciting cortical afterdischarges (AD) 3–5 mA, frequency 8 Hz, duration of stimulation was 15 s. Stimulation was repeated 5 times, always 1 min after the end of previous AD. If no AD was elicited with 5mA stimulation, experiment was terminated. Duration of AD, tendency to generalization and pattern of graphoelements were evaluated. Cortical excitability was tested 19 to 20 hours after the last distilled water (DW) administration; in control animals without any water administration. The smallest number of animals in each group was 8.

Statistical evaluation: duration of AD in individual groups was compared using program GraphPad Prism (ANOVA, t-test; values $p<0.05$ were considered statistically significant).

2. Estimation of water content in the brain

Animals were hyperhydrated with distilled water (Olson *et al.* 1994; Vajda *et al.* 2000; Manley *et al.* 2000; Yamaguchi *et al.* 1997). DW was administered intraperitoneally (i.p.) in the amount corresponding to 20% of the body weight, divided into three parts, injected in 8 hours intervals. Animals were decapitated 19 hours after the last water administration and the water content was estimated by dry/wet ratio (Kamoun *et al.* 2009). Each group consisted of minimum 6 hyperhydrated and 6 control animals. Similarly treated controls did not receive DW.

Results were evaluated using nonparametric Mann-Whitney test, values $p<0.05$ were considered statistically significant.

3. Estimation of natremia

For the standard biochemical estimation of natremia, adult and 25-day-old animals were used, both of the hyperhydrated and control groups.

RESULTS

1. Excitability of cortical neurons

In 12-day-old control animals, duration of the second AD (23.6±1.8 s) was significantly longer than the first AD (17.7±1.5 s; $p<0.0001$). Such prolongation was present also after the next stimulations.

In hyperhydrated animals, AD was evoked only in 3 rats (from 8). With repeated stimulation the seizure duration was always significantly shorter than in controls. No seizure was elicited after the third stimulation (Figure 1).

In 25-day-old control animals the first AD was significantly longer ($p<0.0001$) than the AD after the 2nd, 3rd and 4th stimulation. AD duration after the 5th and 6th stimulation did not differ from the 1st AD.

Tab. 1. Water content in the brain.

	12-day-old	25-day-old	35-day-old
control	85.54%	80.45%	79.48%
hyperhydrated	85.05%*	80.25%*	78.96%*

Control × hyperhydrated; *$p<0.05$

Tab. 2. Natrium plasma level.

	LEVELS	AVERAGE
Adult (n=3)	139.4–140.7 mmol/l	139.9 mmol/l
25-day-old control (n=4)	133.3–137.7 mmol/l	135.7 mmol/l
25-day-old hyperhydrated (n=6)	136.9–139.7 mmol/l	138.3 mmol/l

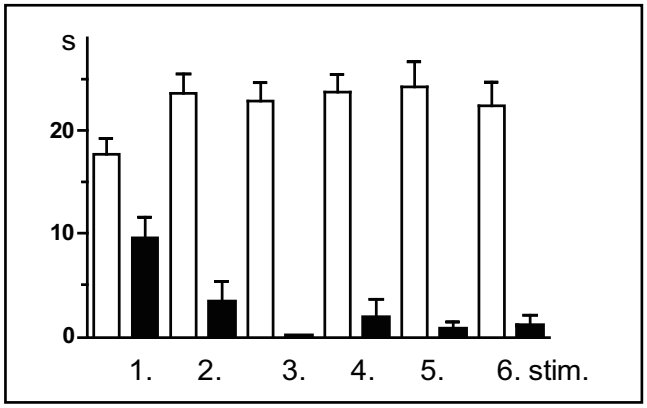

Fig. 1. Duration of AD after the repeated stimulation in 12-day-old animals; Control animals – empty columns; hyperhydrated animals – full columns. Duration of seizures (vertical axis) and succession of stimulation (horizontal axis) are given.

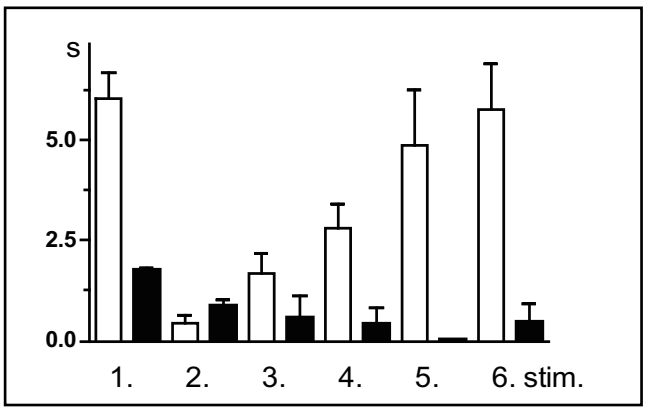

Fig. 2. Duration of AD after the repeated stimulation in 25-day-old animals (legend see Fig. 1).

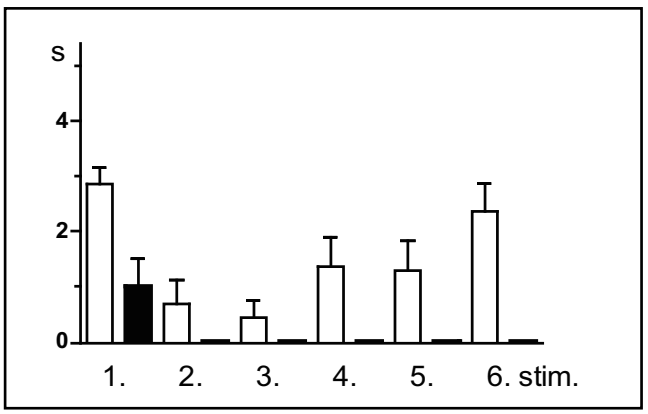

Fig. 3. Duration of AD after the repeated stimulation in 35-day-old animals (legend see Fig. 1).

Two (from 9) 25-day-old animals died after the hyperhydration. From the remaining 7 animals seizures were elicited only in 2 of them. No response came after the 5th repeated stimulation. AD duration was significantly shorter in comparison to controls (Figure 2).

In 35-day-old control animals the first AD was significantly longer (3.9±0.7 s) than the seizure duration after the 2nd, 3rd, 4th or 5th stimulation ($p<0.05$). AD after the 6th stimulation did not differ from that after the 1st stimulation.

One (from 9) 35-day-old animal did not survive the hyperhydration. AD was possible to elicit only in 2 animals. Repeated stimulation triggered no AD (Figure 3).

In case the AD was triggered in the hyperhydrated animal, it was generalized one and its graphoelements were similar to that in controls. In 12-day-old animals, sharp waves appeared; in older animals namely spike-wave complexes were present.

2. Water content in the brain

The expected differences among individual age groups in the brain water content were confirmed (Table 1). Paradoxically, the water content in hyperhydrated animals was in all age groups lower than in controls (*$p<0.05$).

3. Natremia

Natrium plasma level was estimated in healthy adult rats and in 25-day-old hyperhydrated and control rats (Table 2). All measured levels correspond to physiological natremia (Kozniewska & Radomska 2001).

DISCUSSION

Results obtained in the present study revealed that water intoxication achieved by standard hyperhydration method did not increase water content in the brain and the excitability of cortical neurons tested by standard method of cortical stimulation was in young rats significantly inhibited.

Why hyperhydration in young rats did not result in higher water content in the brain has several reasons. In adult rats water intoxication induces cellular oedema with increased water content in brain tissue due to hyponatremia. Thus it is described in the literature (Olson *et al.* 1994; Vajda *et al.* 2000; Manley *et al.* 2000;

Yamaguchi *et al.* 1997) and we have observed similar results in our previous experiments (Kozler & Pokorny 2003). However, standard version of hyperhydration in young rats in our study, did not lead to hyponatremia.

In experimental models with young rats hyponatremia is always induced by vasopressin administration (Silver *et al.* 1999; Timaru-Kast *et al.* 2012) to prevent high mortality of experimental animals during induction of hyponatremia with full dose of water at once. Such a procedure is described by Arieff and co-workers who had, after a single full dose, mortality as high as 84% of experimental animals (Arieff *et al.* 1995). In the present study, we induced hyperhydration by a fractionated application of the water volume corresponding to 20% of the animal's weight. In 25-day-old rats it represented 6 ml of DW administered intraperitoneally with 8-hour intervals between doses. Cortical stimulation and subsequent decapitation followed only after 19 hours after the last dose of DW. We believe that the dilutional hyponatremia did not develop because the dose of water administered intraperitoneally was during the next eight hours excreted from the body in urine. This assumption is supported by findings of the renal AQP2 and vasopressin (ADH) expression immediately after the birth with equals values found in adult rats. With the exception of the first postnatal days when diuresis is controlled by the mother, renal AQP2 and ADH govern the urine flow (Sabolić *et al.* 1995; Zelena *et al.* 2008).

AQP 4 is a key integral protein of brain plasma membranes; in adult rats it serves to the selective flow of water in the brain along to osmolarity gradients between individual compartments (Agre *et al.* 2004). Also the information on the plasma osmolality is mediated by AQP 4, as it is evident from the finding that cells in the central osmoreceptor in hypothalamus are associated via AQP4 with ependymal cells in the subfornical organ and with astrocytes in supraoptic nucleus (Wells 1998). The necessity of AQP4 for the signalling of current osmolarity and for subsequent movement of water in the CNS was highlighted also by other authors (Pasantes-Morales *et al.* 2002; Papadopoulos & Verkman 2007). Expression of AQP 4 is ontogenetically dependent – in young or immature individuals it is lower (Badaut *et al.* 2007; Meng *et al.* 2004). Li and colleagues in their study in mice showed a low expression of AQP4 in the first week of life, a significant increase in the second week with adult values being reached in the fourth week of life (Li *et al.* 2013). Wen and co-workers found a very weak expression of AQP 4 in the first postnatal rats with only 2% of adult values at the age of one week and 25% at age of 14 days (Wen *et al.* 1999). From the above findings it follows that the standard method of hyperhydration cannot induce hyponatremia and a subsequent increase in the amount of water in the brain in young rats, due to much lower expression of AQP 4 than it is in adult rats.

Our results showed a lower water content in brains of hyperhydrated animals compared to control groups. Water intoxication is a serious insult to the homeostasis of internal environment of the brain. Responding to such an insult may be different in young and adult organism. Deghoyan and co-workers described fundamentally different response of rats in various age groups to the exposition to static magnetic field (SMF). SMF exposure leads to the decrease of water content in brain tissues of young animals and an increase in brains of adult and aged ones. Authors report the possible cause of this phenomenon to be age-dependent reciprocal relationship between the expression of the Na/Ca exchanger and the Na/K pump (Deghoyan *et al.* 2014). Although it is a completely different insult, we assume results of that study to represent a parallel to our findings. While in adult rats water intoxication resulted in increased water content in the brain, in young rats the water content in the brain was lower, probably due to ontogenetic immaturity of the active transport systems (Juhászová & Ruscák 1991). The differences in brain water content found in individual age groups of control and hyperhydrated rats confirmed the known ontogenetic decrease of water in the brain tissue (Del Bigio *et al.* 2011).

Excitability of cortical neurons was tested by the repeated stimulation which should induce cortical afterdischarges (AD) (Jandova *et al.* 2012). In control animals of all three age groups, the duration and type of seizure graphoelements corresponded to the literature data (Seifert *et al.* 2010; Fellin & Carmignoto 2004). In all age groups of hyperhydrated animals the excitability of cortical neurons was significantly reduced. It was evident from the findings that AD was elicited only in some of the animals and the seizure duration was always significantly lower than in controls. Excitability of cortical neurons relays on the water and ionic homeostasis in the microenvironment of the brain (Pasantes-Morales *et al.* 2002; Schwartzkroin *et al.* 1998). Hyperhydration can be undoubtedly considered as insult that affects homeostasis. In terms of current knowledge, it can be said that neuronal activity is closely related to glial regulatory functions. One of them is undoubtedly the principle of the so-called tripartite synapse – a coordinated system of pre- and post synaptic neuronal elements with surrounding astrocytic processes. Such tripartite synapse can be certainly influenced by neuronal excitability considering that astrocytes are key cellular elements for maintaining extracellular homeostasis because they control the concentration of ions, neurotransmitters and in general all active molecules. In addition, astrocytes via potassium spatial buffering move excess K^+ back into neurons and because this process is associated with the flow of water it can be concluded that astrocytes grant water homeostasis in the nervous tissue (Tasker *et al.* 2012; Verkhratsky & Parpura 2010). A key structure for movement of water in the brain, as stated above, are AQP 4 expressed in glial elements, mainly in astrocytes. This expression appears to be ontogenetically dependent.

It can be assumed that when the body of young rats with lower expression of AQP 4 is flooded with water, glial regulating mechanisms are not able to maintain the homeostasis, and some of the functions of neuronal circuits, e.g. excitability, fail. Excitability depends not only on the water and ionic homeostasis, but also on the degree maturity of excitatory and inhibitory systems. Their role can be revealed by the decrease of excitability of cortical neurons during development (seizures become shorter), which reduces the risk of synchronous activity – epileptic seizures.

Repeated application of a large volume of distilled water probably brings about an increase of blood volume. This triggers regulatory action via volumo- and baroreceptors. In immature animals it can lead to an „overshoot" reaction and thus reduce the volume of extracellular fluid and disrupt ion homeostasis, which is a prerequisite for maintaining the excitability of cortical neurons. It resulted in the elderly groups, namely in 35-day-old animals, in a significant reduction in activity of neurons especially during repeated functional load.

CONCLUSION

Water intoxication, achieved by standard method of hyperhydratation in young rats (12, 25 and 35-day-old), had different consequences to adult rats. Lower water content in the brain and the absence of hyponatremia in young rats can be explained by lower expression of key integral protein of plasma membranes in the brain – AQP 4. The significant decrease of excitability of cortical neurons in young hyperhydrated rats probably results from inadequate regulatory action of glia – maintenance of ionic microenvironment of the brain. It contributes to the observation that expression of AQP 4 is ontogenetically dependent.

ACKNOWLEDGEMENTS

Supported with grant P – 34/LF 1/7

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