

## SHORT COMMUNICATION

### **Loss of Body Weight Is Accompanying Cellular Brain Edema Induced by Water Intoxication in the Rat**

Petr Kozler, Dana Marešová, Jaroslav Pokorný

Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic

**Short title: Loss of Body Weight Due to Cellular Brain Edema in the Rat**

Corresponding author:

Prof. MUDr. Jaroslav Pokorný, DrSc

Institute of Physiology, First Faculty of Medicine, Charles University

Albertov 5

128 00 Prague 2, Czech Republic

## Summary

Induction of cellular cerebral edema (CE) was achieved by a standard method of water intoxication which consisted of fractionated intraperitoneal administration of distilled water (DW) together with the injection of desmopressin (DP). Using metabolic cage, fluid and food balance was studied in two groups of eight animals: group C – control; group CE – cellular edema induced by water intoxication. For each rat the intake (food pellets and water) and excretion (solid excrements and urine) were recorded for 48 h together with the initial and final body weight. CE animals consumed significantly less food, drank less water and eliminated the smallest amount of excrements. The induction of cellular cerebral edema was accompanied with a significant loss of body weight (representing on average 13 % of the initial values) mainly due to a reduction of food intake. This phenomenon has not yet been reported.

**Key words:** weight loss – water intoxication – brain edema

## Introduction

Brain edema can develop after almost any insult to the brain, including trauma, infarction, neoplasm, or abscess, as well as conditions such as hypoxia, or toxic or metabolic perturbation. In edema, the expanding volume causes increasing pressure, resulting in intracranial hypertension. This life-threatening condition has high rates of mortality in such clinical pathologies, such as stroke and traumatic brain injury, where there's no causal management known at present. The prevailing cause of intracranial hypertension in both above-mentioned pathologies is cellular edema (CE) (Ayata and Ropper 2002, Kimelberg 1995, Kimlberg 2004, Liang et al 2007, Marmarou 2007, Rhoney and Parker 2006).

For studying the pathophysiology of cerebral edema in experimental models, water intoxication is often used (Manley *et al.* 2000, Silver *et al.* 1999, Yamaguchi *et al.* 1997, Yamaguchi *et al.* 1994, Wasterlain and Posner 1968, Papadopoulos and Verkman 2007, Oztaş *et al.* 2000, Plangger and Völkl 1989). We have also been using this method in our experiments (Kozler et al. 2017a, Kozler and Pokorný 2012, Kozler and Pokorný 2014, Kozler et al. 2013, Marešová et al. 2014). In previous studies we found that during the period between the beginning of water intoxication and the experimental analysis, the animals lost their body weight, in average of 13 % of their initial weight. This finding was constant in all

our experiments (in more than 100 rats). The aim of this study was to highlight this phenomenon which was not mentioned by other authors, who used the same experimental method of induction of cellular edema in rats. We analyzed in detail the water and food intake and the excretion of solid excrements and urine in the control and cerebral edema induced rats.

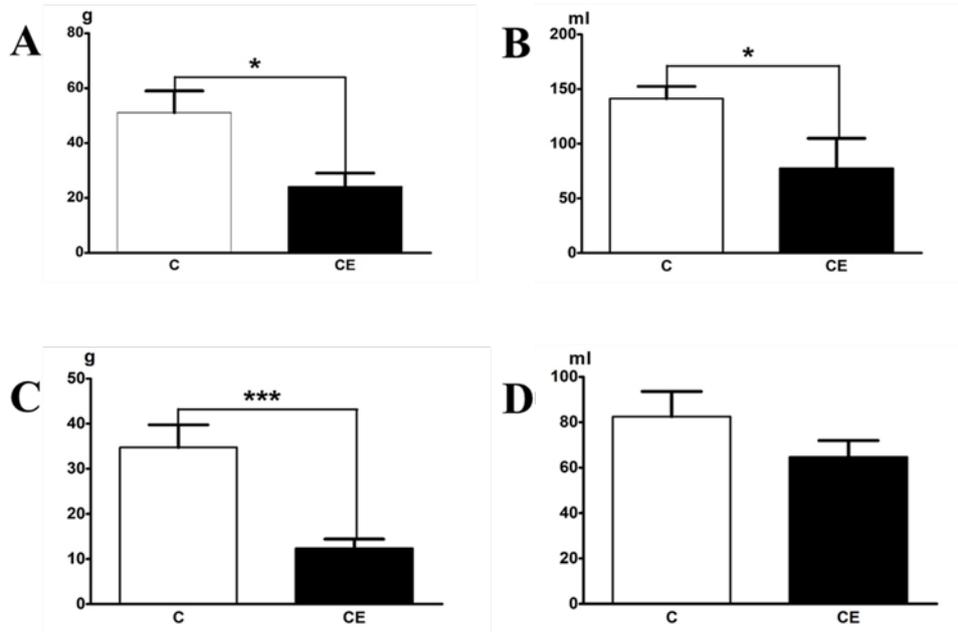
For the experiment, adult male rats of the Wistar strain were used and treated in accordance with the current Guidelines for the Treatment of Laboratory Animals (EU Guidelines 86/609/EEC).

*Cellular edema* was induced by water intoxication, i.e. injecting distilled water in a total amount corresponding to 20 % of body weight intraperitoneally (i.p.) in three consecutive doses over 24 h with a simultaneous administration of desmopressin. Each sub-dose represented one-third of the total dose of 0.032 mg / kg desmopressin (1-desamino-8-D-arginine vasopressin) (OCTOSTIM®, Ferring). Desmopressin is an agonist of vasopressin V<sub>2</sub> receptors the antidiuretic effects of which potentiate the effect of hyperhydration by inducing hyponatremia (Manley *et al.* 2000, Silver *et al.* 1999, Yamaguchi *et al.* 1997).

*Metabolic studies* were performed in rats placed in metabolic cages (Metabolics™, Biological Monitoring) for 48 h, which allowed the accurate measurement of food and fluid intake as well as urine and feces excretion. All rats in metabolic cages had free access to food and water *ad libitum*. For each rat we recorded the amount consumed food pellets and water along with the measurement of excreted solid wastes and urine. The experiment consisted of 16 rats divided into two groups of eight animals: group C – control, intact rats, and group CE – rats with standard CE induction by water intoxication.

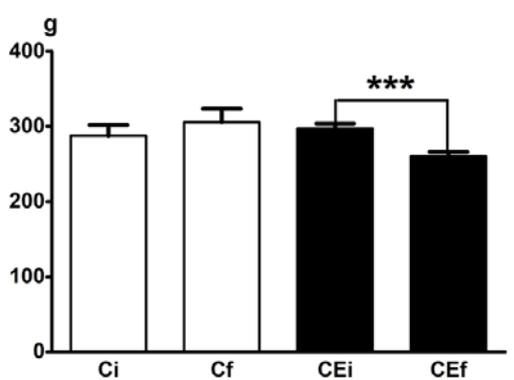
*Statistics* The results of all measurements were statistically evaluated using the GraphPad Prism program (parametric ANOVA and nonparametric Kruskal-Wallis test; statistical significance was set at 5 %).

Our results have shown that the rats with induced cellular edema consumed significantly less food (Fig. 1A), drank less water (Fig. 1B) and produced less solid excrements (Fig. 1C) than the controls. Urine output in rats of both groups was not significantly different (Fig. 1D).



**Fig. 1.** Parameters studied in the control group (C, white columns) and in the group of rats with cellular edema (CE, black columns): **A.** food intake (g/48 h), **B.** peroral water intake (ml/48 h), **C.** solid excrements (g/48 h), and **D.** urine excretion (ml/48 h). Data are means  $\pm$  SEM, significant differences between C and CE groups are indicated as \*  $p < 0.05$ , \*\*\*  $p < 0.01$ .

Figure 2 shows that there was no significant difference between the initial (Ci) and the final (Cf) body weight in the control group (C), but in rats with CE-induction the final weight was significantly lower (CEf) 48 h after the beginning of CE induction. The decrease of body weight in rats with cerebral edema was 12.3 %.



**Fig. 2.** The initial (i) and final (f) body weights (g) in the control group (C, white columns) and in the group of rats with cellular edema (CE, black columns). Data are means  $\pm$  SEM, significant difference between C and CE groups is indicated as \*\*\*  $p < 0.01$ .

Our data show that animals with cerebral edema induced by water intoxication (CE group) lost 12.3 % of their body weight within 48 h. It correlates with significantly lower food intake. Significantly lower delivery of solid excrements in CE-induced animals is associated with their low food intake. These animals were also significantly less drinking. This can be explained by the loss of thirst accompanying the water intoxication. Changes of thirst can be undoubtedly associated with the effect of antidiuretic hormone (desmopressin), which potentiates water intoxication and induces plasma dilution hyponatremia (Sterns 2015). This is a basic prerequisite for cell swelling during the induction of experimental cellular brain edema (Liang *et al.* 2007, Stokum *et al.* 2016). This experimental model shares its pathophysiological mechanisms with the clinical syndrome of inadequate ADH secretion (SIADH), which is also characterized by dilutional hyponatremia (Schwartz *et al.* 1957, Matsuyama *et al.* 2014). Plasma sodium concentration in rats with water intoxication was, on average, 20 mmol/l lower than that in rats of the control group, reflecting a significant degree of water intoxication (Kozler and Pokorný 2003). Since it is at the same time a hypervolemic state, constant urinary excretion is maintained (Wong and Verbalis 2001, Palm *et al.* 2001). That is why there is no significant difference in the urine output in rats of both groups.

It is possible to conclude from the results of our study that there is a direct relationship between body weight loss and reduced dietary intake of rats with cellular edema. In our previous studies, we have demonstrated that the described method of water intoxication actually induces cellular brain edema. There was increased intracranial pressure (Kozler *et al.* 2017a), computer tomography revealed lower brain density compared to normal brain parenchyma (Kozler and Pokorný 2014), and according to dry/wet weight ratio the water content in the brain was also higher in rats with cellular edema (Kozler *et al.* 2013). Other behavioral studies have shown that rats with induced cellular edema have decreased spontaneous locomotor activity (Kozler *et al.* 2017b, Kozler *et al.* 2017c). Low spontaneous physical activity can be one of the factors reducing the food intake. It corresponds with the generally inhibiting effect of CE on the brain activity (Liang *et al.* 2007, Inoue *et al.* 2013).

Our study has shown that the widely used method of water intoxication in experimental models with induced cellular edema in rats leads to a significant loss of body weight. This regularly occurring phenomenon has not yet been reported.

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