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Chapter 7

Effect of Cadmium Contaminated Diet in Controlling Water Behavior by *Meriones shawi*

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Additional information is available at the end of the chapter

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1. Introduction

Body fluid regulation is highly diverse among different animals according to their phylogenic position and the ecological condition [1]. The maintenance of water homeostasis in arid and semi-arid rodent habitats is a critical body function to survive the continually changing environmental condition. The combined effects of anatomical adaptations, behavioural patterns and interactions between hormonal systems allow these small mammals to minimize energetic costs and to finely balance body fluids under a wide range of conditions [2-3]. This is made possible essentially, by homeostatic mechanisms that concentrate urine as an indicator of water regulation efficiency as well as an advantage for colonization and survival [4].

Meriones shawi (Muridae) a semi-desert rodent found in the coastal zone of North-west Africa from Morocco to Egypt [5], has a particular ability to support water restriction until several months [6]. It appears that water intake and water loss are finely balanced by *Meriones shawi*. Water intake was provided from preformed water of food and by metabolic water production as described by King and Bradshaw [7]. Water loss was limited by the production of very dry feces. In addition, *Meriones shawi* produces concentrated urines as results of high plasma concentrations of arginine-vaspressin (AVP) and a large capacity of increasing hypothalamic AVP synthesis and hypophyseal storage [8]. The mean value to concentrate urine in the *Meriones shawi* submitted to water dehydration during 10 days, increased from 1500 mOsm $Kg^{-1}H_20$/ to 3000 mOsm.Kg^{-1} H_20 under laboratory conditions. The maximal capacity to concentrate urine (recorded under laboratory conditions) ranged from approximately 4500 mOsm Kg^{-1} H_20 in the *Meriones shawi* [9]. The alterations of kidney Na-K-ATPase activity, including pronounced heterogeneity of ATPase distributions in nephrons and increased Na-K-ATPase activity in the medullary limb, observed in response to water restriction, can be responsible for this ability [10]. However, AVP is the most

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important hormone to elaborate urines largely hyperosmotic to plasma. A comparative study of water controlling behavior was done between rat laboratory and *Meriones shawi* demonstrated that the level of AVP is 4-fold greater than in dehydrated rats [11]. AVP levels are highly dependent on the state of hydration and correlate with urinary osmolality [12].

AVP or antidiuretic hormone (ADH), is known to be primarily involved in water absorption in the distal nephron of the kidney in mammals. This peptide is synthesized in the soma of hypothalamic magnocellular neurosecretory cells (MNCSs) located in supraoptic (SON) and paraventricular (PVN) nuclei. After water deprivation the axons MNCs project to the neurohypophysis, where Ca^{2+} dependent exocytosis in their nerve terminals causes the release of AVP in blood circulation. The small peptide is secreted by the neurohypophysis in response to increases in plasma osmolality. AVP effects on the renal tubule are mediated by hormone binding to V2 type basolateral receptors coupled trough Gs to adenylyl cyclase and activation of the cyclic adenosine monophosphate - Protein kinase A (cAMP-PKA) cascade [13]. The hydroosmotic action causes a dramatic increase in the osmotic water permeability of connecting cells, principal cells and inner medullary collecting duct cells. The result is highly concentrated urines produced in response to water restriction.

The success of rodent to survive harsh environment condition goes back to several years ago. However, these animals are faced to substantial anthropogenic threats due to the introduction of heavy metals in environment in the last decades. Cadmium (Cd), a nonessential heavy metal, is widely distributed in the environment due to its use in primary metal industries and phosphate fertilizers [15, 16]. Food and cigarette smoke are the biggest sources of Cd exposure for the general population [17]. In humans, Cd exposure leads to a variety of adverse effects and contributes to the development of serious pathological conditions [18-19] linked to enhanced aging process as well as cancer [20-21]. Cd produces also neurotoxicity with a complex pathology [22-23]. In animals, Cd was shown to be toxic to all tissues such as liver [24], reproductive organs including the placenta, testis and ovaries [17, 25]. Several studies in some industrial sites in Tunisia showed that some habitats of *Meriones shawi* became contaminated by Cd [26-27] *Meriones shawi* have accumulated cadmium on different organs particularly on kidney and liver. It has been reported that kidneys, which play a major role in hydro-mineral maintenance, are considered to be the organ that is most sensitive to Cd, depending on exposure dose, time and administration route [28]. Several studies indicated that the main critical effect of cadmium exposure is kidney dysfunction. Excretion of low molecular weight proteins is characteristic of damage to the proximal tubules of the kidney. The increased excretion of low-molecular weight proteins in the urine is a result of proximal tubular cell damage [29]. This raises the possibility that body fluid homeostasis and vasopressinergic system could be subtly disrupted by Cd exposure. In this study, we were interested to determine whether Cd naturally incorporated in food would alter the water balance in *Meriones shawi* who appears to show a remarkable physiology flexibility of water regulation in both time and space. Effects of Cd exposure upon the water-conserving abilities of this specie were assessed through measurements of water metabolism (total body water (TBW), water influx (Fin), water efflux (Fout) and water turnover rates (WTR) under differing water availabilities. Water fluxes were determined by direct analysis following the principles

described by Holleman and Dieterich [30]. Cd effects in the brain were also determined by immunohistochemistry in the supraoptic (SON) and paraventricular (PVN) nuclei at the control level of the central AVP which is the most important hormone in the regulation of water balance in mammals.

2. Material and methods

2.1. Animals and housing conditions

All experiments were carried out on adult male of Muridae; *Meriones shawi* [31] originating from the south of Tunisia. The rodents were captured from non-polluted regions and kept in captivity in our breeding facility for two generations. The animals were put in single cages and housed in an air-conditioned room maintained at 25 ± 1°C at a relative humidity of 45 ± 10 %, with a 12 h dark-light cycle. The diet of the control group consisted of granular flour mixed with distilled water at the dose of 1 L /1.5 Kg of granular flour. Contaminated diets of treated animals consisted of granule flour mixed with a solution of cadmium chloride ($CdCl_2$) at dose (1 g Cd/1L H_2O/1.5 kg of granule flour) [32]. Food was given in the form of balls dried at 60 ° C for 72 hours. Water was supplied *ad libitum*.

Animals were randomly selected and divided into four groups. Eight animals, the first goup was used as control (C). Water was given *ad libitum*. Meriones of the second group (8 animals) received the same diet but were deprived of water (D-). The third group was treated with Cd in the form of $CdCl_2$ (Cd) at dose (1 g Cd/1L H_2O/1.5 kg of granule flour). The last group was also treated with $CdCl_2$ at the same dose but was deprived water D+Cd.

For immunohistochemistry study, treatment period had lasted from eight days to two weeks. Each animal was put in a metabolic cage for eight days in order to collect feces and 24 h urine each day at the same time. Urine samples were collected on paraffin oil to prevent evaporation and measured in mL/day. Daily consumption of drinking water and food of each group were measured throughout the study. It was not possible to collect urine since the 10 days of dehydration.

All of the protocols were carried out in accordance with French standard ethical guidelines for laboratory animals (agreement 75-178, 5_16_2000).

2.2. Techniques

Body weight of each animal was determined throughout the experiment. Blood samples were collected from the infra-orbital sinus into heparinized hematocrit capillary tubes, immediately before the experimental period and eight days later. These samples were centrifuged at 1500 g x for 10 min in order to determine hematocrit. At the end of experimentation rodents were sacrificed by decapitation, and brain, kidneys and livers were immediately removed and weighed. The weight of organs (%) was calculated as g /100 g of body weight. Finally these organs were dried at 60° C and weighed for the determination of dry weight.

2.3. Determination of water fluxes

Water fluxes were determined by direct analysis following the principles described by Holleman and Dieterich [30]. Rates of water flux represent the loss of water via excretion and evaporation and the simultaneous input of water, via metabolic water production and pre-formed water via food and drink (Nagy and Costa 1980). Free water content of the food determined by drying to constant weight at 60 °C was 3 %. The metabolic water content was determined from carbohydrate, fat and protein composition [33]. Thus 1 g of given food contains 0.509 mL of water. The intact unshaven carcasses were sublimated to dryness. The difference between live and dry weight was taken as total body water (TBW).

After determining urine volume and feces weight, urine samples were frozen at -30 °C while the feces were dried for 72 h. Water efflux was calculated as the difference between the influx and total body water. Water fluxes are expressed in H_2O mL per day. Finally these fluxes were normalized to the average body weights and expressed in $kg^{-0.82}$. In small mammals an allometric relationship exists between the water efflux or influx and body weight (W) in kilograms, which is $Fin=K.W^{0.82}$ ([34-35], expressed as mL/day/100 g body weight.

2.4. Tissue preparation

Meriones were anesthetized with sodium pentobarbital (70 mg/kg, i.p; Sanofi, Libourne,France) and perfused transcardially with heparin in physiological saline, followed by 500 mL of a freshly prepared solution of 4 % (wt / vol) paraformaldehyde in phosphate – buffered saline (PBS ; pH = 7.4). The brains were rapidly removed and postfixed overnight in 4 % paraformaldehyde at 4 °C. Forty micrometer thick coronal sections were cut with a Vibratome (VT 1000S; Leica, Nussloch, Germany). Brain sections were collected in cold PBS.

2.5. Immunohistochemistry

Free-floating sections were pretreated for 20 min with 3 % hydrogen peroxide in PBS to quench endogenous peroxidase. They were then washed with PBS (3 x 10 min), preincubated for 90 min at room temperature in PBS containing 0.05 %. Triton X-100 and 3 % normal horse serum. Sections were incubated for 36 h at 4 °C with Mouse anti-AVP antibody (1: 5000 dilution).

After incubation, sections were rinsed extensively with PBS (four times, 15 min) and incubated for 1.5 h in a 1/100 dilution of biotin conjugated horse anti-goat antibody and other secondary antibodies. Texas Red conjugated rabbit anti-mouse antibody (1/200; dilution; Jackson ImmunoResearch). For amplification, we used tyramide signal amplification fluorescence system technology (NEN, Boston, MA, USA). For details see Banisadr et al. [36]. After washing, sections were mounted onto gelatin-coated slides in Vectashield (Vector) and observed on fluorescent microscope (BX61; Olympus, Melville, NY) and a connected image-acquisition software (Analysis) was used.

2.6. Statistical analysis

Data are shown as the mean ± SEM. All results were compared to control animals (C), as well as to the Cd-exposed animals (Cd). For all our experiment, a two-way ANOVA was used to analyze the differences between groups, followed by a Dunnett's test with a threshold of significance of $p < 0.05$ and $p < 0.01$ to detect specific differences, using a statistical software package (XLSTAT version 2009.1.1).

3. Results

3.1. Body mass

During the eight days of experimentation, body mass doesn't change significantly in the control group. Body weight loss represented 5.77 ± 0.05 % in *Meriones* treated with Cd (expressed in % of initial body weight). A higher significant increase in body weight loss (16 ± 0.19 % of initial body weight) was observed following 8 days of water restriction. The body weight loss (19.34 ± 0.29 %) is greater in the *Meriones* group both water-deprived and treated with Cd.

3.2. Relative weights of organs

Relative weight of liver in controls is an average of 0.05 ± 0.01. Cd exposure significantly altered the relative weight of liver (0.036 ± 0.01) following eight days of treatment. Water restriction had no effect on relative weight of liver as compared to control *Meriones*.

Decrease in relative weight of liver was also observed in water-deprived group and simultaneously treated with Cd. No differences were found in relative kidney weights (6.8 ± 0.9) in all groups under all experimental conditions.

3.3. Food consumption

Consumption of food was expressed per 100 g of body weight. Control animals consumed an average of 4.5 g/day of food. There was a significant ($p < 0.01$) decrease of food intake in the Cd-exposed group (2.54 ± 0.2 g daily). Food intake of the water deprived groups was similar to that of the controls. When water deprivation was combined with Cd exposure, the decrease in food intake became larger and statistically significant compared with both control ($p < 0.01$) and Cd-exposed groups ($p<0.05$).

3.4. Hematocrit

After eight days of experimentation, hematocrit (44.32 ± 1.08 %) did not change significantly in any treatment condition as compared to day 1 (Fig. 3).

3.5. Water metabolism

Water metabolism data are shown in Table 1.

Treatment	Initial body weight (g)	Total body water (mL)	Total body water (%W)	Water influx mL	Water efflux mL	Water influx ml $Kg^{-0.82}$ d^{-1}	Water efflux mL.$Kg^{-0.82}$ d^{-1}	WTR in (% body water d^{-1})	WTR out (% body water d^{-1})	Urinary osmolality mOs/kg H_20	Plasma osmolality (mOs/kg H_20)
Control	117.44 ±3.66	62.97 ±2.55	55.79 ±2.74	10.90 ±3.63	10.27 ±3.66	63.83 ±22.70	60.16 ±22.79	17.36 ±6.44	16.37 ±6.43	1100 ± 2	307.6 ± 4.2
Cd-exposed Meriones	134.41 ±19.37	61.09 ±5.28	48.38 ±5.87	10.04 ±3.08	9.34 ±3.04	50.50 ±11.12	47.11 ±11.53	15.51 ±4.55	14.43 ±4.52	1600 ± 1.9**	332 ± 3
Deprived water Meriones	120.37 ±16.85	64.89 ±1.23	61.30 ±9.28	◎◎ ** 2.17 ±0.23	◎◎ ** 1.93 ±0.56	◎ ** 12.48 ±1.27	◎ ** 11.96 ±3.34	◎◎ ** 3.18 ±1.06	◎◎ ** 3.12 ±0.67	◎◎ ** 1700 ±1.9	345 ± 3
Deprived water and Cd-exposed Meriones	128.25 ±18.67	67.59 ±1.36	60.50 ±9.99	◎◎ ** 1.73 ±0.50	◎◎ ** 1.81 ±0.76	◎ ** 9.32 ±2.11	◎ ** 9.62 ±3.28	◎◎ ** 2.45 ±0.73	◎◎ ** 2.66 ±1.09	◎◎ ** 1162 ±2	307.6 ± 4.2

Table 1. Effects of Cd exposure on water metabolism (Total Body Water, Water influx, Water efflux, and Water Turnover Rates (WTR)and urinary and plasma osmolalities) in adult *Meriones shawi* male under hydrated or deprived water conditions. Data are expressed as mean ± SEM from 6 animals in each group. ** p <0.01significantly different from controls C.◎ p<0.05; ◎◎p<0.01 signifficantly different from Cd-exposed Meriones.

Total body water content in control group was 55.79 ± 2.74 (expressed by % of body weight). Throughout the experiments, body water was not significantly altered in any group. In animals having free access to water, water enters through metabolic water production and pre-formed water via food and drink.

The value of water influx was 10.90 ± 3.63 ml/ 63.83 ± 22.79 ml.$Kg^{-0.82}$ .d^{-1}. This water influx (Fin) was not significantly affected in the group treated with Cd in comparison to control group. The loss of water via excretion (urine and fecal) and evaporation was Fout =10.27 ± 3.66 ml/60.16 22.79 ml.$kg^{-0.82}$.d-1. Water fluxes rate were equal (Fin = Fout). This indicates that animals were in water equilibrium. After, one week of Cd exposure, water flux rates

were not significantly affected in the group treated with Cd in comparison to control group and water equilibrium was maintained throughout the experiment.

Following one week of dehydration, the water influx rates was significantly decreased from about 5 times in Meriones treated or not with Cd ($p<0.01$). Cd exposure may not affected the water intake during our experiment.

In spite variations in water intake in different experimental conditions, all animals were in water equilibrium where water influx (Fin) and efflux (Fout) rates were equal (Fin = Fout).

3.6. Distribution of immunohistochemical staining for AVP

In control *Meriones shawi*, AVP immunostaining was found to be homogeneously distributed in the large magnocellular neurons of SON (Fig. 2) and PVN (Fig. 3). In agreement with previous, in the absence of Cd ingestion, there was a significant compensatory increase in AVP immunostaining by the SON of deprived animals following eight days of water restriction (Fig. 2C) and two weeks (Fig. 2D) compared to controls animals (fig 2A and B). This increase in AVP immunostaining was also observed in PVN respectively after eight days and two weeks of water restriction (Fig. 3C) and (Fig. 3D) compared respectively to controls animals (fig 3a and B).

Similarly to what was observed for AVP immunostaining in deprived animals without Cd, AVP immunoreactivity is strongly increased in SON following eight days of water restriction (Fig. 2E) and PVN (Fig. 2F) compared to controls animals respectively (Fig.2A) and (Fig.3A). The increase of AVP immunostaining became more important by prolonged experiment for two weeks respectively in SON (Fig. 2F) and PVN (Fig. 3F).

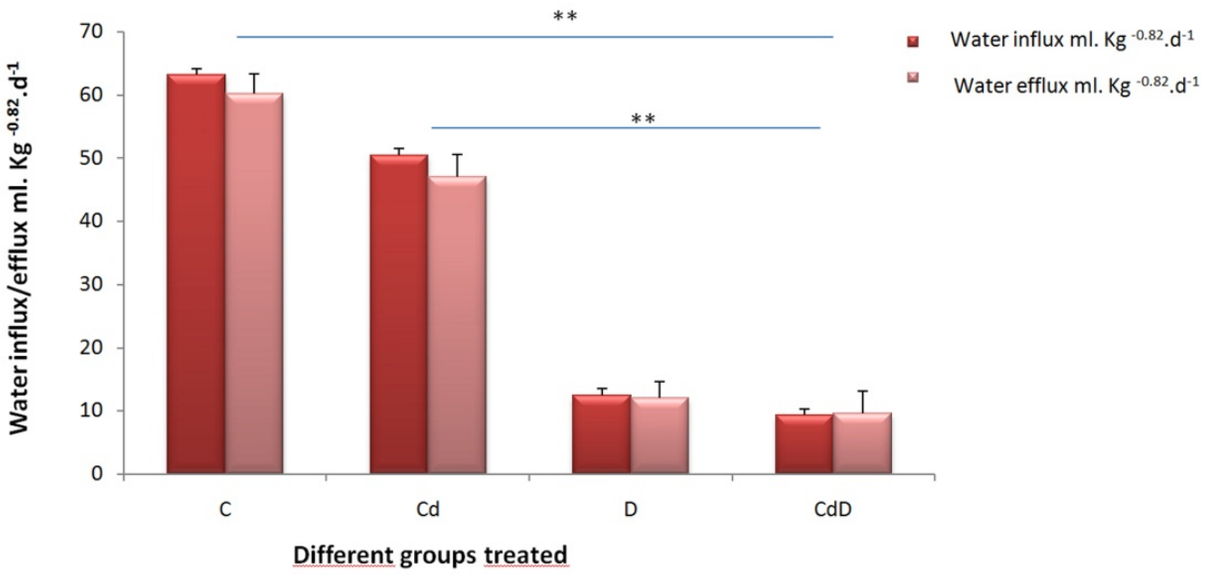


Figure 1. Effects of Cd exposure on water Water influx and efflux in adult *Meriones shawi* male under hydrated or deprived water conditions. Data are expressed as mean ± SEM from 6 animals in each group.

However, AVP immunostaining from deprived water animals in the presence of Cd was markedly and significantly lower in SON (Fig. 2G) than in deprived water animals but not treated with Cd for a week (Fig. 2C). This decrease of AVP immunostaining becomes more important following two weeks of treatment (Fig. 2H) in comparison in two weeks deprived water animals not treated with Cd (Fig. 2D). Similar effect of AVP depletion in SON was also observed in PVN in simultaneously deprived water group and Cd-exposed Meriones during eight days (Fig. 3G) and two weeks (Fig. 3H) in comparison to those eight days deprived water group and two weeks deprived water groups and not treated with Cd.

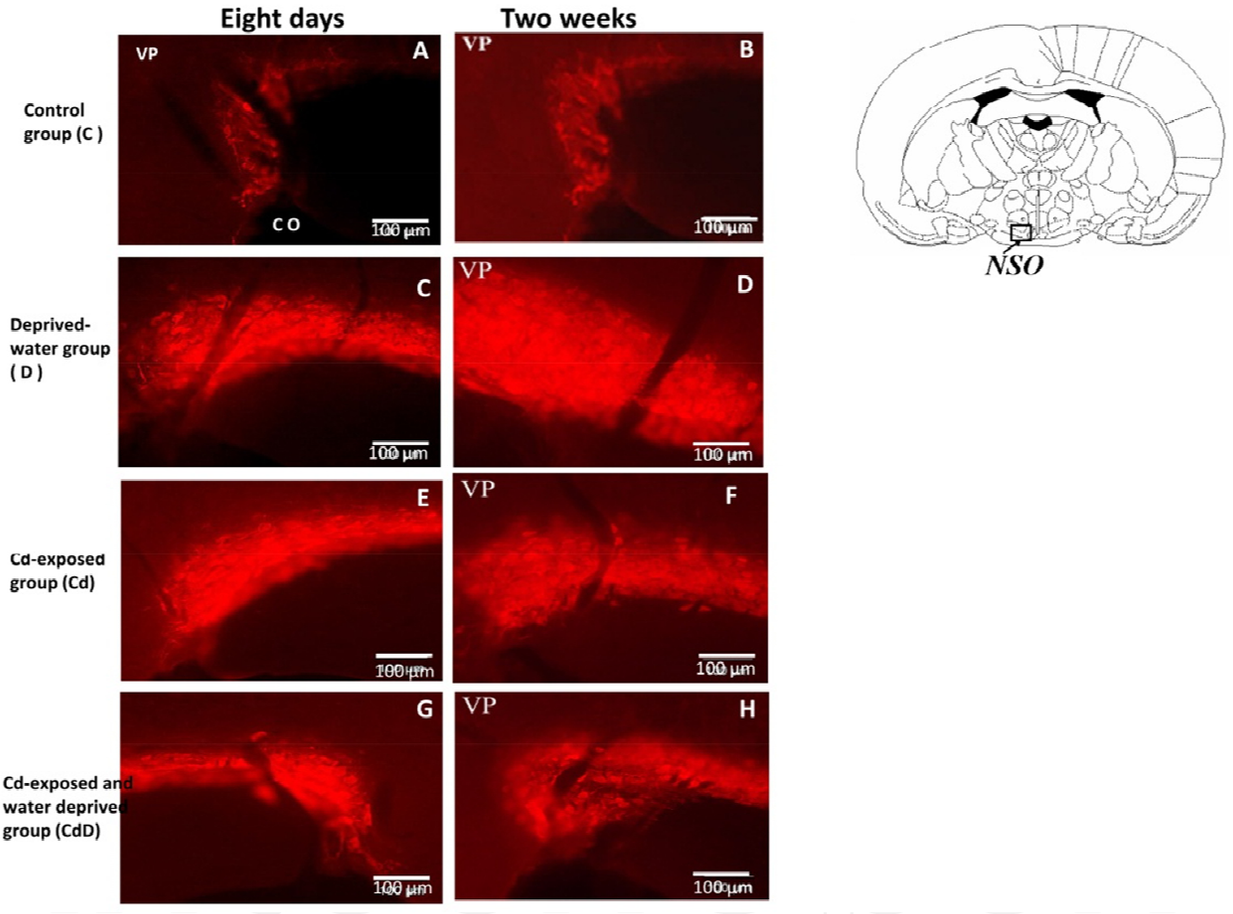


Figure 2. Effect of Cd exposure on AVP immunoreactivity distribution in the hypothalamic supraoptic nuclei (NSO) in *Meriones shawi.* Control group (A,B) , eight days deprived-water group (C), two weeks deprived-water group (D), Eight days Cd-exposed group E, two weeks Cd-exposed group (F), 8 days Cd-exposed and also deprived water group (G), two weeks Cd-exposed and also deprived water group (H).Water deprivation increased the immunohistochemical signal in SON nuclei (C); this increase became more important following two weeks of water deprivation (D) . Similar effect was observed when Meriones are exposed to Cd following one week (E) and two weeks (F). However, Exposure to Cd causes a decrease in immunoreactivity of vasopressin at SON by Meriones deprived water for a week (G) compared to those water deprived group but not treated with Cd (C). This decrease was also observed after two weeks of treatment (H) as compared to water deprived Meriones (D). Scale bars =100 µm.

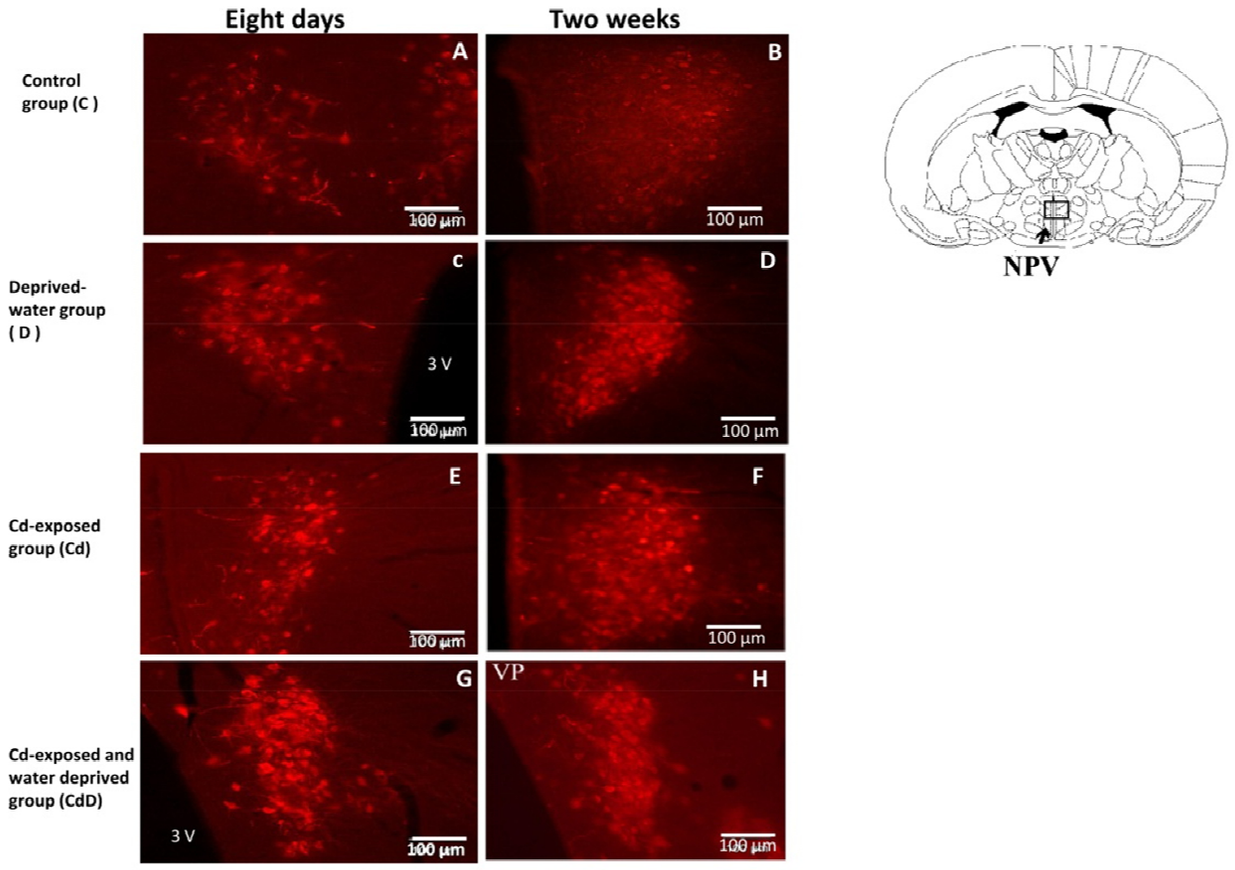


Figure 3. Effect of Cd exposure on AVP immunoreactivity distribution in the hypothalamic paraventricular nuclei (NPV) in *Meriones shawi.* Control group (A,B) , eight days deprived-water group (C), two weeks deprived-water group (D), Eight days Cd-exposed group E, two weeks Cd-exposed group (F), 8 days Cd-exposed and also deprived water group (G), two weeks Cd-exposed and also deprived water group (H).Water deprivation increased the immunohistochemical signal in NPV nuclei (C); this increase became more important following two weeks of water deprivation (D) . Similar effect was observed when Meriones are exposed to Cd following one week (E) and two weeks (F). However, Exposure to Cd causes a decrease in immunoreactivity of vasopressin at NPV by Meriones deprived water for a week (G) compared to those water deprived group but not treated with Cd (C). This decrease was also observed after two weeks of treatment (H) as compared to water deprived Meriones (D). Scale bars =100 µm.

3.7. Effect of Cd on water metabolism

Meriones shawi, success dry and wet seasons by stimulating anti-diuretic and diuretic systems alternately. The maintenance of tonicity of body fluids by within a very narrow physiological range is made possible by well-developed homeostatic mechanisms that control the intake and loss of water [2, 37]. This capacity was also observed when *Meriones shawi* was treated with Cd under various conditions of water deprivation. *Meriones shawi* are able to maintain body water (55.79 %) status under water deprivation conditions. The absence of change in hematocrit value observed by deprived water groups treated or not with Cd (45%) suggests that regulatory processes occur, resulting in the maintenance of body water content and increase in urine concentration [38-39]. Whether in nature or under

laboratory conditions, control groups were in water equilibrium (water influx = water efflux) [32]. The value of water influx was 10.90 ± 3.63 ml/ 63.83 ± 22.79 ml.$Kg^{-0.82}$ .d^{-1} (figure 1). This water influx (Fin) was not significantly affected in the group treated with Cd in comparison to control group. The loss of water via excretion (urine and fecal) and evaporation was Fout =10.27 ± 3.66 ml./60.16 ± 22.79 ml.$kg^{-0.82}$.d^{-1}. Water fluxes rate were equal (Fin = Fout). This indicates that animals were in water equilibrium. After, one week of Cd exposure, water flux rates were not significantly affected in the group treated with Cd in comparison to control group and water equilibrium was maintained throughout the experiment. Following one week of dehydration, the water influx rates was significantly decreased from about 5 times in Meriones treated or not with Cd ($p<0.01$). Cd exposure appears not to impair this capacity during our experiment. However in water deprived animals there was a lower rate of water influx and efflux compared to controls. This low rate of water influx and efflux was similar in water deprived animals and treated with Cd simultaneously (water metabolism are shown in table 1).

The urinary osmolality (UO) in the control *Meriones* group was around 1100 mOsm.Kg^{-1}.H_20. The mean value increased significantly from 1100 mOsm $Kg^{-1}H_20$/ to 1600 mOsm.Kg^{-1} H_20 following one week of water restriction. This value not change when animals were exposed to Cd [40]. The plasma osmolality (PO) was around 270 mOsm.Kg^{-1}. It was not changed in all groups following one and two weeks of experiment. Hematocrit was around (44.32 ± 1.08 %). It did not change in any treatment condition as compared to day 1. All these results are shown in table 1.

In spite of the variations in water metabolism, all animals were in water equilibrium, at the end of experimentation. All these results indicate that even under the most stringent conditions *Meriones shawi* has a strong capacity to maintain a homeostasis state. It seems evident that water restriction induced a pronounced body mass loss in animals after eight days of treatment without available drinking water. This indicates a depletion of reserves of endogenous metabolic water supplies as an alternative to fresh water [9]. Although changes in body mass in Cd-exposed animals are assumed to be due to reduction of daily consumption of food, this decrease of food uptake became larger in animals both deprived water and treated with Cd. This is in agreement with the findings of Pettersen et al. [41] who demonstrated that rats exposed to Cd become anorexic. An important finding in this study, described by other authors Woltowski et al. [42] and Leffel et al. [43] was the early occurrence of Cd induced hepatic damage manifested by lower liver weight, which was explained by the high level of Cd found in livers of exposed animals. As shown by Sudo et al. [44] Cd preferentially localizes in hepatocytes after administration, and its concentration may exceed the capacity of intracellular constituents, mainly metallothioneins (MTs) to bind Cd [45]. MT-bound Cd then appears in the blood plasma [44] and is efficiently filtered through the glomeruli, and subsequently taken up by the tubules leading to its accumulation in kidney [46-47].

In order to maintain physiological serum osmolality, water intake and water loss are finely balanced by *Meriones shawi* even under water restriction and Cd exposure condition (fig 1). It appears that *Meriones shawi* are able to retain water by excretion of highly

concentrated urine [8]. Water loss was also limited by the lowered faecal water loss achieved by the production of very dry feces. In deprived water Meriones we show that water intake was provided from preformed water of food and by metabolic water production as described by Speakman [48] and King and Bradshaw [49]. Our findings are in agreement with previous reports showing that renal concentrating mechanisms are the first line of defense against water depletion [4, 12, 50]. It is well established that modifications of serum osmolality during depletion are detected via osmoreceptors by magnocellular mainly located in the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN) in the brain [39, 51]. These neurons increase their electrophysiological activity during water restriction leading to an increase of AVP synthesis [52- 53] (and facilitates sustained antidiuresis [54] (De Mota et al. 2004). In contrast to what was observed in the laboratory rat where dehydration causes a dramatic depletion of hypothalamic AVP immunoreactivity in both SON and PVN [55- 56], water restriction induced in our model an increase in expression of AVP. This increase becomes more important with time of restriction water.

Interestingly, the ability of acute systemic dehydration to produce AVP in both SON and PVN in *Meriones shawi* deprived water and not treated with Cd, was also observed while treating Meriones with Cd but not deprived water. We hypothesized that potential effects of Cd might include exaggerated synthesis of AVP during Cd exposure in our model *Meriones shawi* and support the idea of an increase of AVP as result of Cd intoxication (see figure 2 and 3). These findings suggest that Cd ingestion has potential effects on the vasopressinergic system that responds with elevated synthesis of AVP under stimulated conditions [57]. A large number of studies have demonstrated that Cd exposure produce marked neuroendocrine changes in animals [58- 59] and human [60].

The current study is the first to explore the potential impact of Cd exposure on the magnocellular neuroendocrine system responsible for hydromineral balance. In this paper, we shown an involvement of the hypothalamo-vasopressinergic system of AVP, wish plays a fundamental role in the maintenance of body fluid homeostasis, in the protective reactions of the organism during Cd exposure in *Meriones shawi* by secreting arginine-vasopressin in response to a variety of physiological stimuli, including osmotic [61-63] and nonosmotic stimuli [64, 65]. In support of this, we found that water metabolism was identical in both groups of deprived water *Meriones* and treated *Meriones* with Cd respectively. In contrast, the adaptive response of vasopressin enhancement secretion in both SON and PVN under stimulated conditions as dehydration or Cd exposure in *Meriones shawi,* was attenuated in Meriones simultaneously exposed to Cd and dehydration of water, as compared to deprived water but not treated with Cd group. Our results show an inhibitory effect of Cd exposure on AVP immunoreactivity in both SON and PVN in response to acute water restriction in adult male *Meriones.* We hypothesized that potential effects of Cd might modifies vasopressinergic system which is amplified under water restriction, where AVP neurons are under constant stimulation and suggested that vasopressinergic system is subtly disrupted. Similar effect of AVP depletion in both SON and PVN, produced by Cd ingestion in deprived water was also

observed in deprived water laboratory rats treated by an organochlorine pollutant (polychlorinated biphenyls (PCBs) during 15 days [66]. According to these authors, the AVP decline was attributable to specific effects of overt toxicity and/or malaise oral of PCBs on vasopressinergic hypothalamic cells function. In combination with the efficacy of *in vitro* application, these data are consistent with direct actions on components of the hypothalamo-neurohypophysial system present within the SON [67-68]. PCBs has been reported to inhibit nitric oxide synthase activity. It is noteworthy that the inhibition of nitric oxide production in SON tissue punches produces a virtually identical, selective effect on dehydration stimulated intranuclear AVP release *in vitro* [69] and has been reported to exaggerate pituitary depletion of AVP in the intact deprived water rat [70] .

Most strikingly, vasopressin is recognized as circulating hormone. Its actions were essentially confined to peripheral organs. However, currently AVP have been shown to be released in the brain as chemical messengers. AVP, like many peptides, when released within the brain, plays an important role in social behaviour. In rats, AVP is implicated in paternal behaviors, such as grooming, crouching over and contacting pups. AVP is also important for partner preference and pair bonding, particularly for males in a variety of species. It has been shown that AVP has powerful influences on complex behaviours [71]. Disruption of vasopressinergic system has been linked to several neurobehavioural disorders including prader-Willi syndrome, affective disorders, obsessive-compulsive disorder and polymorphisms of V1a vasopressin receptor have been linked to autism [72].

4. Conclusion

On the basis of the current study, we conclude that Cd exposure modifies the vasopressinergic neuronal system and provides information regarding the neurotoxicity risks that this element presents for mammals and human populations exposed to Cd even to low amounts without affecting directly water metabolism. We are currently trying to study the linkage between Cd exposure and water controlling behavior at different level of the central nervous system.

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