Acta Histochemica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.com/locate/acthis

The underlying physiological basis of the desert rodent *Meriones shawi's* survival to prolonged water deprivation: Central vasopressin regulation on peripheral kidney water channels AQPs-2

A. Elgot[a,b], O. El Hiba[a,c], M. Belkouch[d], H. Gamrani[a,*]

[a] Equipe de Neurosciences, Pharmacologie et Environnement, Université Cadi Ayyad (UCAM), Faculté des Sciences Semlalia, B.P. 2390, Marrakech, Morocco
[b] Unité des Sciences biomédicales, Laboratoire des Sciences et Technologies de la Santé, Institut Supérieur des Sciences de la santé, Université Hassan 1er Settat, Morocco
[c] Equipe des Physiopathologies Nutritionnelles, laboratoire de Biochimie, Nutrition et Valorisation des Ressources Naturelles, Université Chouaib Doukkali, El Jadida, Morocco
[d] Université de Lyon, Inserm UMR 1060, UMR Inra 1397, CarMeN Laboratory, IMBL, INSA-Lyon, Villeurbanne, France

ARTICLE INFO

Keywords:
Meriones shawi
Dehydration
Immunohistochemistry
Kidney
AQP-2

ABSTRACT

Meriones shawi (M. shawi) is a particular semi-desert rodent known by its resistance to long periods of thirst. The aim of the present investigation is to clarify the underlying mechanisms allowing M. shawi to resist to hard conditions of dehydration. For this reason we used two different approaches: i) a morphometric study, which consists in measuring the effect of dehydration on body and kidneys weights as well as the report kidney weight/body weight, ii) By immunohistochemistry, we proceed to study the effect of dehydration on the immunoreactivity of central vasopressin (AVP) and the kidney aquaporin-2 (AQP-2) which is a channel protein that allows water to permeate across cell membranes. Our results showed both a body mass decrease accompanied by a remarkable kidneys hypertrophy. The immunohistochemical study showed a significant increase of AQP-2 immunoreactivity in the medullar part of Meriones kidneys allowing probably to *Meriones* a great ability to water retention. Consistently, we demonstrate that the increased AQP-2 expression occurred together with an increase in vasopressin (AVP) expression in both hypothalamic supraoptic (SON) and paraventricular nucleus (PVN), which are a major hub in the osmotic control circuitry.

These various changes seen either in body weight and kidneys or at the cellular level might be the basis of peripheral control of body water homeostasis, providing to *M. shawi*a strong resistance against chronic dehydration.

1. Introduction

Without water, humans can only survive a few days, water is essential for the diffusion of metabolites to the cells, to regulate body temperature and many other complex metabolic processes. The desert species living in dunes, and dry areas; they have developed mechanisms to promote water conservation, which allows them to avoid, tolerate or control the excess heat. Terrestrial animals lose water through perspiration, respiration and the excretion of urine. Many small desert animals, before rejecting air breathing, they refresh it in their nostrils and condense water that it contains. Moreover, kidneys of desert mammals have a powerful ability to concentrate urine, so they excrete their urine with less water compared to non-desert species (Fielden et al., 1990). As a desert-adapted rodent, *M. shawi* is characterized by its resistance to long periods of thirst that could reach several months (Laalaoui et al., 2001; Elgot et al., 2009, 2012; Gamrani et al., 2011). Moreover, this rodent presents many characteristics and adaptations allow it to survive in dry and hostile seasons, for example, *M. shawi* nephrons are much hypertrophied, allowing it effectively to retain water by excretion of highly concentrated urine (Rabhi et al., 1996). Otherwise, cellular hypertrophy associated with dehydration is considered as a compensatory phenomenon that enables to kidney to economize water; this hypertrophy is strongly associated with blood glucose increase (Wolf and Ziyadeh, 1999; Sartorelli and Fulco, 2004; Neubauer, 2007). Moreover, in our laboratory and unlike in rat, it has been shown that supraoptic nucleus of hypothalamus which is a brain structure involved in the control of hydrous balance is well structured in this rodent compared to rat (Gamrani et al., 2011; Elgot et al., 2012) allowing it to better resist to dehydration. Therefore, dehydration, is a well known nutritional problem, it can be defined as depletion in total

* Corresponding author.
E-mail addresses: gamrani54@gmail.com, gamrani@uca.ac.ma (H. Gamrani).

https://doi.org/10.1016/j.acthis.2017.11.006
Received 24 August 2017; Received in revised form 15 November 2017; Accepted 17 November 2017

body water content due to pathologic fluid losses, diminished fluid intake, or a combination of both (Gross et al., 1992), and poses particularly acute dangers for body cells. To adapt against dehydration, urine concentration allows desert rodent to better retain water and to cope to severe osmotic conditions, for this reason, their kidneys have developed specialized integral membrane protein channels that facilitate the efficient permeation of water across the biomembranes named aquaporin-2 (AQP-2). Thus, it serves as a major water channel responsible for the concentration of urine (Fushimi et al., 1993; Nielsen et al., 1993, 1995).

To clarify how *M. shawi* regulates body water and resist to severe conditions of dehydration, and to better understand the urine-concentrating process under thirst challenge, we proceed to study by immunohistochemistry the effect of prolonged water deprivation on the expression of AQP-2 in kidney. Further, immunohistochemical analysis was used for determining hypothalamic AVP expression in vasopressinergic neurons of the SON and PVN in response of different states of water deprivation.

2. Materials and methods

2.1. Animals

Experiments were carried out in *M. shawi*, captured around the semi-desert regions of Marrakesh and kept in captivity in our breeding farm for several generations. Animals of 1 month of age of both sexes were used for the immunohistochemistry study including 5 controls (free drinking water) and 12 dehydrated animals divided in 4 groups (3 animals each): 1 month, 2 months, 3 months, and 10 months dehydrated groups.

The rodents were housed at a constant room temperature (25 °C), with a 12 h dark–light cycle and free access to food to all studies groups. Water bottles have been given freely to controls but not to water-deprived animals. All animals were treated in compliance according to guidelines of the Cadi Ayyad University, Marrakech (Morocco) with adequate measures undertaken to minimize pain and animal discomfort.

Kidneys of *M. shawi* used in the entire study are served to draw the kidneys weights histograms.

2.2. Immunohistochemistry

After anesthesia with sodium pentobarbital (40 mg/kg i.p.), all animals were perfused transcardially with chilled physiological saline and paraformaldehyde (4%) in phosphate buffer (PBS, 0.1 M, pH 7.4, Riedel-de Haen, Seelze, Germany). Brains and kidneys were post-fixed in the same fixative for 12 h at 4 °C, dehydrated in graded ethanol solutions (70–100%), passed through serial polyethylene glycol solutions: PEG (Merck-Shuchardts, Hohenbrunn, Germany, Cas No. 25322-68-3) and embedded in pure PEG. Frontal sections (20 µm) were cut with microtome, collected and rinsed in PBS to wash out the fixative. Sections were performed throughout the supraoptic and paraventricular nuclei of hypothalamus and also the medulla part of the kidneys. Free floating sections were incubated in vasopressin antibody (1:1000, Chemicon International, Temecula, CA, USA, cat #AB1565) and aquaporin-2 antibody (cat #KP9201, lot #34574,Calbiochem, USA) diluted respectively in 1/1000 and1/2000 in PBS containing 0.1% Triton X-100 and 1% bovine serum albumin: BSA (Sigma-Aldrich, St Louis, USA, CAS No. 9048-46-8, Lot. 078K0729). After three washes in the same buffer, sections were incubated for 2 h at room temperature in goat anti-rabbit biotinylated immunoglobulins (1/2000, Vector Labs, Burlingame, CA, USA, cat# BA-1100, lot. WO611) and then, after washing, incubated in streptavidin peroxidase (1/2000; Vector Laboratories Burlingame, Californie, USA, cat# PK-6101). Peroxidase activity was revealed by incubating sections in 0.03% DAB (3-3,diaminobenzidine, Sigma-Aldrich; Oakville, Canada, CAS No. 868272-85-9) in 0.05 M Tris buffer, pH 7.5, containing 0.01% H2O2. The sections were then collected, dehydrated and mounted in Eukit for optic microscopy observation. The specificity of the immunoreactivity was tested following the subjection of the slides to same immunohistochemical protocol described above by either using the preimmune serum or omitting of the primary antibodies. These tests showed that the primary antibodies used against AQ2 displays specific labeling.

2.3. Immunolabelling quantification and statistical analysis

The quantification of the immunorectivity was performed according the protocol published by Vilaplana and Lavialle (1999) (Vilaplana and Lavialle 1999). Briefly, the digitization and storage of images were performed using a Zeiss-Axioskop 40 microscope fitted with a Canon digital camera. Images were digitized into 512 × 512 pixels with eight bits of grey resolution and were stored in TIFF format. Image processing and quantification were performed using Adobe Photoshop® v.6.0 (Adobe Systems, San Jose, CA, USA). After transformation of each image to the binary mode, the percentages of black pixels were obtained using the image histogram option of Adobe Photoshop®. This percentage corresponds to the AVP and AQP-2 immunopositive areas of both the hypothalamic regions and the medulla part of the kidney. For controls and water-deprived animals. Different samples were chosen from each slide (field of AVP or AQ2 expressions), a total of 5 slides from each animal were quantified and then an average of 3 animals (considered as repetitions) is presented for each studied group. Data are reported as mean ± SEM, and were subjected to a one-way analysis of variance (ANOVA). Post hoc differences between group means were tested with the Tukey test. Values of p lower than 0.05 were considered significant. Statistical analyses were performed using the computer software SPSS 10.0 for Windows® (IBM, Chicago, IL, USA).

3. Results

3.1. Effect of dehydration on body weight

As expected, at the end of the 10 months of water deprivation, animals have a lower weight than before the challenge. Indeed, we found a change in body weight starting from the first month of experimental dehydration. Meriones lost, on average, 20% of their body weight over the 1-month experiment. Gradually as dehydration is increasing there is a significant decrease in body weight which reaches its minimum in the interval between 6 and 10 months of water deprivation. Moreover, animals that have been treated by reverse rehydration during a period of one month, have known a significant recovery of body weight (Fig. 1A), (*p < 0.05).

3.2. Effect of dehydration on kidney weight

Our results showed that the report kidney weight/body weight of Meriones increases gradually and reaches the peak after 10 months of dehydration (Fig. 1B). At this stage; kidneys of this desert rodent are hypertrophied and increased size (Fig. 1C), (*p < 0.05).

3.3. Immunohistochemistry of renal water channel aquaporin-2 (AQP-2)

In controls, the results obtained after application of the aquaporin-2-antibody (anti-AQP-2) on sagittal sections (Fig. 2a) of Meriones kidneys display a slight immunostaining dispersed in the medulla. Moreover, we note that the immunolabelling of the Malpighian pyramids cells is scattered and homogeneous which extends longitudinally and centripetally from the basal part of the pyramids to the calyx (Fig. 2A). After one month of dehydration, the AQP-2 immunoreactivity show a slight difference compared to control. Thus, the slights display reactive alignments along the renal pyramids and tend to be grouped into specific centripetal fascicles (Fig. 2B). After 2 months of dehydration, we