Mathematical Biosciences 244 (2013) 176–187

Contents lists available at SciVerse ScienceDirect

Mathematical Biosciences

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

Regulatory volume decrease of rat kidney principal cells after successive hypo-osmotic shocks

Sotirios G. Zarogiannis [a,*], Alexander V. Ilyaskin [b], Galina S. Baturina [b], Liubov E. Katkova [b], Dmitriy A. Medvedev [c,d], Denis I. Karpov [c,d], Alexander P. Ershov [c,d], Evgeniy I. Solenov [b,d]

[a] Department of Physiology, Medical School, University of Thessaly, Biopolis, Larissa, Greece
[b] Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[c] Lavrentyev Institute of Hydrodynamics Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia
[d] Novosibirsk State University, Novosibirsk, Russia

ARTICLE INFO

Article history:
Received 2 July 2012
Received in revised form 9 May 2013
Accepted 10 May 2013
Available online 31 May 2013

Keywords:
Cell volume regulation
Osmotic stress
Osmoregulation
Kidney
Mathematical modeling

ABSTRACT

Outer Medullary Collecting Duct (OMCD) principal cells are exposed to significant changes of the extracellular osmolarity and thus the analysis of their regulatory volume decrease (RVD) function is of great importance in order to avoid cell membrane rupture and subsequent death.

In this paper we provide a sub-second temporal analysis of RVD events occurring after two successive hypo-osmotic challenges in rat kidney OMCD principal cells. We performed experimental cell volume measurements and created a mathematical model based on our experimental results. As a consequence of RVD the cell expels part of intracellular osmolytes and reduces the permeability of the plasma membrane to water. The next osmotic challenge does not cause significant RVD if it occurs within a minute after the primary shock. In such a case the cell reacts as an ideal osmometer. Through our model we provide the basis for further detailed studies on RVD dynamical modeling.

1. Introduction

The renal collecting tubule is the final component of fine control for salt and water homeostasis in all mammals. Thus, it is the key regulator of the extracellular volume and solute composition of the organism. For the most part this process is mediated and regulated through the principal cells of the outer medullary collecting duct (OMCD) that are the main cell population in this part of the kidney epithelium [35]. The importance of this cell population to the whole body homeostasis is critical and evident from the fact that the hereditary disorder Nephrogenic Diabetes Insipidus is associated with a water channel (Aquaporin 2; AQP2) of the principal cells, while Liddle's syndrome is associated with a sodium transporter (amiloride sensitive epithelial sodium channel; ENaC) of the principal cells [2,20].

Since the final composition of the urine is determined in the collecting duct, the cells comprising it are exposed continuously to hyposmotic and hyperosmotic conditions. Therefore it is critical for these cells to maintain their viability and functionality. As a consequence they have adaptive mechanisms regarding their cell volume regulation. The hyposmotic environment causes cell swelling that can disrupt the cell membrane if not counterbalanced. The adaptive mechanism of recovery to cell swelling is termed regulatory volume decrease (RVD) [18]. The series of events in RVD is loss of intracellular osmolytes followed by a rapid concomitant efflux of intracellular water. Thus, the pivotal counterparts of RVD are ion conducting pathways, mainly permeable to K^+, Cl^- and organic anions leading ions out of the cell and water transporting proteins (AQP's) allowing efflux of water along the osmotic gradient [10,18,22,31,33].

Renal principal cells are highly permeable to water since they express three types of AQP's, AQP2 apically and AQP3/AQP4 basolaterally [27]. Water enters cells through the lumen via apical AQP2 and exits to the circulation via basolateral AQP3 and AQP4 [27]. Hypotonicity challenge has been reported to decrease the translocation of AQP2 to the cell surface in order to protect it from excess swelling [34].

Swelling of collecting duct cells may occur very fast and repetitively. Although several attempts have been made to model the RVD of various components of kidney epithelia, little is known about the behavior of cells in conditions of repetitive osmotic changes. Moreover, there are no studies regarding swelling and RVD in cells with fast kinetics of these processes recorded with sub second time resolution. Most mathematical models involve slow exchange processes or steady state modeling of ion and water

* Corresponding author. Address: Department of Physiology, Medical School, University of Thessaly, Biopolis, 41110 Larissa, Greece. Tel.: +30 2410685558; fax: +30 2410685555.
E-mail address: szarog@med.uth.gr (S.G. Zarogiannis).

0025-5564/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.mbs.2013.05.007

transport across the cell membrane [1,7,14,15,19,25,39,40]. We have already reported a mathematical modeling approach of fast RVD in kidney epithelia, only for a single hypo-osmotic shock [32], but no modeling approach exists for describing the cell behavior after sequential osmotic shocks. The most adequate way to study such processes is the combination of experimental measurements and mathematical modeling.

The purpose of this work was to study the time course of cell volume changes in principal cells of rat kidney OMCD in hypotonic medium, with high temporal resolution. We provide evidence regarding the changes of ion and water transport governing this cell behavior. Moreover, based on these experiments we opted to construct a mathematical model of this reaction in order to elucidate sub-second cell volume kinetics and RVD processes.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the Ethical Committee of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences. Wistar rats weighting 150–200 g (Breeding Laboratory of Experimental Animals, Institute of Cytology and Genetics, Novosibirsk, Russia) were kept in individual cages and received standard diet. For standardization of animals' state and increase of the osmotic water permeability of the OMCD epithelial cells prior to beginning of the experiments, rats were subjected to anti-diuresis by water deprivation and receiving only dry food for 36 h (hypo-hydrated animals).

2.2. Solutions

The solutions used were based on PBS (137 mM NaCl, 4.7 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$, 300 mOsm/l, pH = 7.4) containing 1.0 mg/mL glucose. This solution was chosen in order to be able to degas it without affecting its pH. To create osmotic challenges, bath solutions were changed from normal to PBS diluted with distilled water (1:1). Two subsequent hypotonic challenges were performed with 10–15 s lag.

For medullary substance dispersion calcium-free PBS was used (137 mM NaCl, 4.7 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4, 0.5 mM $MgCl_2$, 0.05 mM $CaCl_2$).

2.3. Collecting duct fragments

Rats were anesthetized by pentobarbital (50 mg/kg intraperitoneally) and decapitated. Extracted kidneys were placed in ice-cold PBS (pH 7.4), then de-capsulated and de-corticated. A suspension of collecting duct fragments was prepared. Tissue from the outer medulla zone was squeezed through a needle (0.45 mm i.d.) in the ice-cold calcium-free PBS. The resulting suspension was filtered through a nylon mesh, diluted 10 times with Eagle MEM culture medium and centrifuged (100 g, 10 min, 4 °C). The sediment containing the tubules was diluted with culture medium to an appropriate concentration of about 10 fragments per μL. This suspension was used in experiments as a preparation of OMCD fragments.

In experiments with hypotonic shock suspension of OMCD fragments was incubated in 0.5 × PBS at 37 °C during 3 min.

2.4. Perfusion chamber and microscopy

A superfusion chamber was constructed as an acrylic block mounted on the objective of an upright microscope (water immersion 65× magnification, numerical aperture 1.1, thermal stabilization at 36.8 ± 0.2 °C). The temperature was chosen so as to provide adequate conditions for all transporters and to prevent inhibition of transporters with high energy of activation that could happen at low temperatures [6].

The flow rate of the perfusate was 20 mL/min, which resulted in complete solution exchange in less than 100 ms. Fluorescence measurements of cell volume were performed by the calcein quenching method as it was previously described [30]. Cell volume changes were expressed as relative values of calcein fluorescence. Fragments of outer medullary collecting duct (OMCD) were placed on a glass plate and were loaded with Calcein-AM (Invitrogen, CA, USA) (5.0 μM) by incubation for 15 min at 4 °C, and then for 25 min at 37 °C in 5% CO_2. The glass plate with the fragments of the OMCD was positioned on the stage of a microscope (LOMO-R8, St. Petersburg, Russia). Calcein fluorescence was measured continuously with a halogen light source, through a calcein filter set (480 nm excitation, 490 nm dichroic mirror, 535 nm emission), a photomultiplier detector with a pinhole diaphragm in order to be able to select the cells of interest at the end of the fragment where the both apical and basolateral surface of the cells are exposed to bath solution, and with a 14-bit analog-to-digital converter PCL-818HG (Advantech). The data acquisition rate was 10 ms.

Calcein fluorescence was calibrated by the simultaneous measurements of the fluorescence and the changes of cell height of superfused OMCD fragments challenged by various osmolality of the extracellular medium (150, 300, 400 and 600 mosmol/kgH_2O). The superfusion chamber was mounted on the stage of Zeiss Observer Z1 microscope (Zeiss, Germany) (63×, oil immersion, N.A. 1.4) and CCD camera AxioCam HSm (Zeiss, Germany) (frame rate for data acquisition was 10 fps). On the basis of 3D confocal reconstructions in solutions of different osmolalities (see Appendix C), the assumption that OMCD cell volume is proportional to cell height cubed could be accepted: $V/V_0 = (H/H_0)^3$. It is evident from the results of the confocal reconstructions that the osmotically inactive part of the cell volume does not exceed 10% of the total cell volume and thus is small enough to be neglected. The according calibration plot is shown in Fig 1.

2.5. Statistics

Averaged experimental recordings of calcein fluorescence ($n = 5$) were presented as mean value and standard error of mean (M ± SEM).

2.6. Methods of mathematical modeling

In order to construct our model we consider a non-polarized, non-excitable cell expressing water, potassium, sodium, chloride and organic osmolyte channels, Na/K-pump and KCC and NKCC

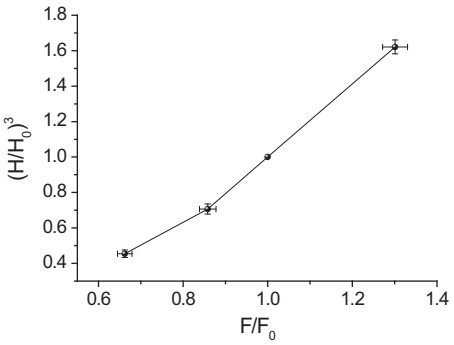

Fig. 1. Calibration plot for the Calcein quenching method. Abscissa: relative Calcein fluorescence; ordinate: relative cell height cubed. All values are presented as M ± SE.