Available online at www.sciencedirect.com
ScienceDirect
Zoology 111 (2008) 287–294
ZOOLOGY
www.elsevier.de/zool

# Volume regulation mechanisms in *Rana castebeiana* cardiac tissue under hyperosmotic stress

Laura N. Cruz, Marta M. Souza*

*Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Estadual de Londrina, CEP 86051-990, Londrina, PR, Brazil*

Received 16 April 2007; received in revised form 14 June 2007; accepted 11 July 2007

## Abstract

Volume changes of cardiac tissue under hyperosmotic stress in *Rana catesbeiana* were characterized by the identification of the osmolytes involved and the possible regulatory processes activated by both abrupt and gradual changes in media osmolality (from 220 to 280 mosmol/kg $H_2O$). Slices of *R. catesbeiana* cardiac tissue were subjected to hyperosmotic shock, and total tissue $Na^+$, $K^+$, $Cl^-$ and ninhydrin-positive substances were measured. Volume changes were also induced in the presence of transport inhibitors to identify osmolyte pathways. The results show a maximum volume loss to $90.86 \pm 0.73\%$ of the original volume (measured as 9% decrease in wet weight) during abrupt hyperosmotic shock. However, during a gradual osmotic challenge the volume was never significantly different from that of the control. During both types of hyperosmotic shock, we observed an increase in $Na^+$ but no significant change in $Cl^-$ contents. Additionally, we found no change in ninhydrin-positive substances during any osmotic challenge. Pharmacological analyses suggest the involvement of the $Na^+/H^+$ exchanger, and perhaps the $HCO_3^-/Cl^-$ exchanger. There is indirect evidence for decrease in $Na^+/K^+$-ATPase activity. The $Na^+$ fluxes seem to result from $Mg^{2+}$ signaling, as saline rich in $Mg^{2+}$ enhances the regulatory volume increase, followed by a higher intracellular $Na^+$ content. The volume maintenance mechanisms activated during the gradual osmotic change are similar to that activated by abrupt osmotic shock.


***Keywords:*** Amphibians; Heart cells; Osmotic stress; Osmolytes; Ion transporters

## Introduction

Cell volume is not only important in defining any given cell's external morphology and internal osmolality, but it also affects cellular functions such as membrane transport, metabolism, migration, growth and cellular death (Wehner et al., 2003). A cell in an environment subject to osmotic variations may increase or decrease its volume when under hyposmotic or hyperosmotic challenge, respectively. In order to eliminate the extra volume gained from exposure to a hyposmotic medium, it is necessary for a cell to release solutes (inorganic and/or organic osmolytes) and water. To regain volume lost through exposure to hyperosmotic media, a cell will take up solutes, which is also followed by water. During both types of volume regulatory processes, membrane transport mechanisms are activated (Hoffmanm and Dunham, 1995; Lang et al., 1998).

Although the majority of amphibians are aquatic or semi-terrestrial, they may be found in diverse environments and also inhabit arid regions (Schmidt-Nielsen, 2002).

*Corresponding author. Tel.: +55 43 3371 4650; fax: +55 43 3371 4467.
*E-mail address:* mmsouza@uel.br (M.M. Souza).

0944-2006/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.zool.2007.07.013

An important amphibian characteristic is that they possess damp, permeable skin, which is highly vascularized and participates in breathing and osmoregulation (Pough et al., 1996). Their skin exhibits an evaporative rate similar to free water (Bentley, 1966; Bentley and Yorio, 1979; Hillyard, 1999). Bentley and Yorio (1979) state that in general these animals do not drink water to rehydrate, as this may lead to osmotic stress.

A principal problem for animals that occupy arid environments is evaporative water loss through the body surface. As amphibian urine is very dilute, it may be utilized as a water storage system, but when this is exhausted, and before the animals can activate endocrinal regulation (Rocha and Branco, 1998; DeGrauw and Hillman, 2004), they might well face a dehydration state which promotes an osmotic and ionic concentration of the animal's body fluids (Hillyard, 1999; Schmidt-Nielsen, 2002). This concentration of fluids results in a hyperosmotic stress on different animal tissues. Concerning cell volume, an interesting environmental response has been observed in amphibian freeze tolerance studies which show a correlation between plasma glucose levels and red blood cell volume (Steiner et al., 2000).

Although amphibians are classically known as models of ionic transport in different epithelia (Ussing and Zerahn, 1951), there are no published studies on ionic transport systems associated with cardiomyocyte volume regulation in these animals.

Volume regulation studies in general are performed by submitting cells to an abrupt change in medium osmolality. This probably does not occur in natural environments (Ordaz et al., 2004). Perhaps better models of *in vivo* conditions were first developed by Lohr and Grantham (1986) in the kidney proximal tubule, where cells were exposed to a gradual decrease in medium osmolality. It has been purported that the osmotic shock induced by a gradual change in osmolality may activate regulatory mechanisms different from those activated by a large, single-step change in osmolality (Van Driessche et al., 1997; Souza et al., 2000).

Thus, it is important to evaluate volume changes in amphibian cardiac tissue under hyperosmotic stress, considering a relevant shock that the animals would be expected to experience in their natural habitat. Also, our purpose is to address the osmolytes involved, and the possible regulatory processes activated, by both abrupt and gradual changes in media osmolality.

## Material and methods

### Experimental model – the animal

*Rana catesbeiana*, the North American bullfrog, has been introduced into Southern Europe, South America, and Asia, and is now widely distributed. Bullfrogs are very adaptive to different environments, and can tolerate high temperatures. A bullfrog may bury itself in the mud for protection during adverse conditions and to prevent dehydration (Conant, 1975). The species was chosen for this study because it is an animal that faces a broad range of environmental conditions and is relatively large (~0.2 kg, length ranging from 90 to 150 mm), which indicates a relatively large heart.

Cardiac tissue from *R. catesbeiana* (Amphibia, Anura) was chosen for its excitable characteristics, as excitable cells must balance specific ionic requirements for their normal function, with ionic transport necessary for volume regulation. Specimens of *R. catesbeiana* were anesthetized by ice cold water, and their hearts were removed. The ventricle was divided into thin slices of approximately 85 mg (~1 mm thickness) and transferred to isosmotic saline (220 mosmol/kg $H_2O$), before any of the experimental procedures were performed.

### Experimental solutions

The control saline (220 mosmol/kg $H_2O$, ~pH 7.1) consisted of 111.2 mmol NaCl, 1.4 mmol $CaCl_2$, 1.9 mmol KCl, 2.4 mmol $NaHCO_3$, 3.0 mmol dextrose. Hyperosmotic salines were prepared by concentrating the control solution by 20%, 30% and 50% (265, 280 and 335 mosmol/kg $H_2O$, respectively).

### Cellular volume estimation

Ventricle slices were first incubated for 15 min in a petri dish containing control saline and then carefully blotted on a filter paper, weighed on a balance (1 mg accuracy, Gehaka, BG-200, Brazil) and then immersed in one of the hyperosmotic salines (265, 280 or 335 mosmol/kg $H_2O$). Following the osmotic shock, the slices were weighed as described above in 15 min intervals. Wet weight changes induced by hyperosmotic conditions were followed for 2 h, and then for 1 h after returning the tissues to control conditions. We compared the above results with the *expected* volume change of the cardiac tissue if it were to display osmometric behavior $\pi_C/\pi_E$, according to van't Hoff's law, where we considered $\pi_C$ as the osmolality of the isosmotic saline and $\pi_E$ as the osmolality of the hyperosmotic saline.

In order to evaluate the precision of the wet weighing method used to estimate changes in volume, some ventricle slices were weighed every 15 min for 2:30 h in isosmotic saline (220 mosmol/kg $H_2O$) as a control. During this period the volume did not change by more than 1.7%. This same procedure has been used in previous studies (Souza and Scemes, 2000; Amado et al., 2006). Any cellular damage resulting from slicing the tissue would not influence our comparisons since it