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Relationship between extracellular osmolarity, NaCl concentration and cell volume in rat glioma cells

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Abstract. The cell volume, which controls numerous cellular functions, is theoretically linearly related with the inverse osmolarity. However, deviations from this law have often been observed. In order to clarify the origin of these deviations we electronically measured the mean cell volume of rat glioma cells under three different experimental conditions, namely: at different osmolarities and constant NaCl concentration; at different NaCl concentrations and constant osmolarity and at different osmolarities caused by changes in NaCl concentration. In each condition, the osmolarity was maintained constant or changed with NaCl or mannitol. We showed that the cell volume was dependent on both the extracellular osmolarity and the NaCl concentration. The relationship between cell volume, osmolarity and NaCl concentration could be described by a new equation that is the product of the Boyle-van't Hoff law and the Michaelis-Menten equation at a power of 4. Together, these results suggest that in hyponatriemia, the cell volume deviates from the Boyle-van't Hoff law because either the activity of aquaporin 1, expressed in glioma cells, is decreased or the reduced NaCl influx decreases the osmotically obliged influx of water.

Key words: Ion channels — Aquaporins — Tonicity

Introduction

Extracellular tonicity, and the associated cell volume regulation, control many molecular and cellular functions including gene expression, neuromediator and hormone release, cell proliferation, apoptosis and migration (Van der Kloot and Molgo 1994; Soroceanu et al. 1999; Lang et al. 2000; Najvirtova et al. 2003; Rouzaire-Dubois et al. 2004; Jeon et al. 2006; Ernest et al. 2008; Okada et al. 2009). Independently of protein synthesis, the cell volume (V) is dependent on the external osmolarity and is theoretically described by the Boyle-van't Hoff law:

$$\frac{V}{V_0} = \left[\left(\frac{V-b}{V_0}\right)\frac{\pi_0}{\pi}\right] + \frac{b}{V_0} \quad (1)$$

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where V_0 and π_0 are isotonic volume and osmotic pressure, respectively, and b is the osmotically inactive volume. Except in erythrocytes that behave as a perfect osmometer over a large range of osmolarity (Prickett et al. 2008), deviations from the Boyle-van't Hoff law have often been observed in various cell types. First, depending on cell types and external ion composition, b/V_0, which is obtained by extrapolation, varied between 0 and 0.8 (Arrazola et al. 1993; Crowe et al. 1995; Rouzaire-Dubois et al. 1999, 2009). Second, in hyposmotic solutions, the cell volume deviated from the perfect osmometer and saturated at low osmolarity (Arrazola et al. 1993; Rouzaire-Dubois et al. 2009). Third and independently of the osmolarity, the cell volume was dependent on the NaCl concentration (Rouzaire-Dubois et al. 1999, 2009).

During anisotonic challenges, deviations from the perfect osmometer can be explained by a regulatory volume decrease (RVD) or increase (RVI) due to ion and organic osmolyte fluxes mediated by the activation of channels and transporters and osmotically obliged water fluxes (Lang et al. 1998). However, even after inhibition of these volume regulations, the cells remained non-perfect osmometers during hyposmotic challenges (Rouzaire-Dubois et al. 2009).

In order to clarify these problems, we measured the volume of C6 rat glioma cells under three different conditions: 1) at different osmolarities and constant NaCl concentration; 2) at constant osmolarity and different NaCl concentrations; 3) at different osmolarities due to changes in NaCl concentration. From these experiments, we developed a new equation that described the change in cell volume as a function of both extracellular tonicity and NaCl concentration.

Materials and Methods

The experiments were performed on C6 rat glioma cells at temperatures of 20–22°C. The cells were cultured as previously described (Rouzaire-Dubois et al. 2004, 2009). The cells were trypsin-detached, pelleted at 100 × *g* for 5 min, resuspended and mechanically stirred in isotonic or anisotonic solutions. Two main isotonic solutions were used containing either 160 mM NaCl (solution 1) or 80 mM NaCl + 150 mM mannitol (solution 2). Third set of isotonic solutions was obtained from solution 2 by changing the concentration of both NaCl and mannitol. The osmolarity of all these solutions was identical to that of the culture medium (338 ± 0.02 mOsm·kg^{-1}). Anisotonic solutions were obtained by changing the concentration of either NaCl in solution 1 or mannitol in solution 2. All solutions contained (in mM): KCl 5, $MgCl_2$ 2, $CaCl_2$ 1, Hepes 10, pH adjusted to 7.4 with NaOH. The osmolarity of solutions was determined using an osmomat 0.30 freezing point osmometer (Fisher Bioblock Scientific, Illkirch, France). In each individual experiment, the mean cell volume of 20 000 to 70 000 cells was determined with a Z2 Coulter channelyser (Beckmann-Coulter, Villepinte, France). Absolute cell volume was obtained using beads (Coulter) as standards. Chemical reagents were from Sigma (Saint Quentin Fallavier, France). In each condition, cell volumes were expressed as the mean ± the standard error of the mean (SEM) of *n* experiments. The SEM was often smaller than 2% and, consequently, the SEM bars in the figures were often smaller than the symbols. Parameters of Eqs. (2) and (3) were determined with built in regression routing in the Microcal Origin 7 software (OriginLab, Northhampton, MA, USA).

Depending on the NaCl concentration, the absolute cell volume in isotonic or isonatriemic solutions decreased by 3 to 13% in 10–15 min (see Figs. 1A, 2A and 3A). Between 13 and 15 min, the final cell volume (V') was expressed relative to the cell volume at time 0 (V_0), calculated by exponential regression of the change in cell volume with time in isotonic or isonatriemic conditions. It should be noted that V_0 corresponds to the cell volume in culture medium. In anisotonic or anisonatriemic solutions the final cell volume between 13 and 15 min was also expressed relative to V_0. In each experiment, the absolute cell volume was subsequently measured on the same cultures in control and test solutions. Under these conditions, Eq. (1) becomes:

$$\frac{V}{V_0} = \left[\left(\frac{V'-b}{V_0}\right)\frac{\pi_0}{\pi}\right] + \frac{b}{V_0} \quad (2)$$

where b/V_0 is the relative osmotically inactive volume after 13–15 min incubation in test solutions.

Results

Cell volume at different osmolarities and constant NaCl concentration

In this series of experiments, the solutions contained 80 mM NaCl and different concentrations of mannitol. Fig. 1A

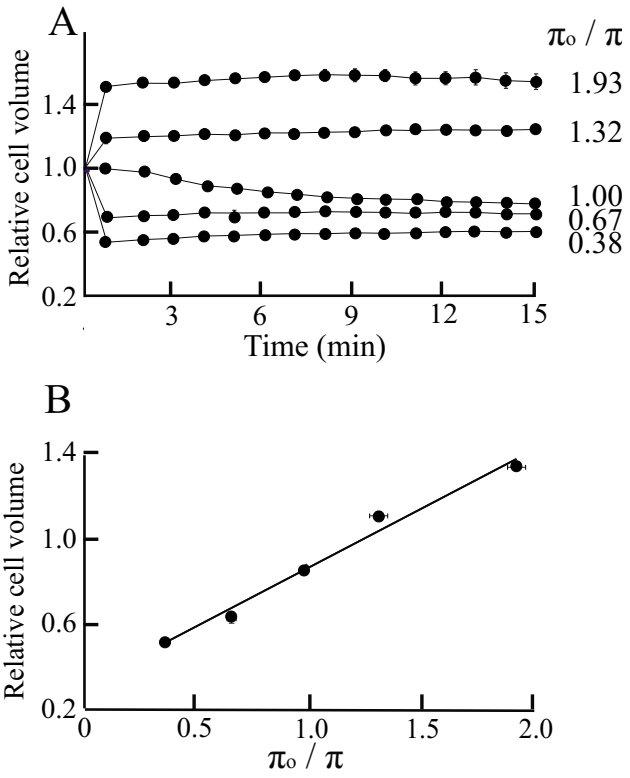

Figure 1. Relative cell volume as a function of the inverse relative osmolarity at constant NaCl concentration. **A.** Evolution with time of the relative cell volume at different osmolarities where π_0 and π are the isotonic and anisotonic pressure, respectively. The osmolarity was changed with mannitol at constant (80 mM) NaCl concentration. **B.** Mean relative cell volume between 13 and 15 min as a function of the relative reciprocal osmolarity according to Eq. (2). The straight line was a linear regression through the points. The isosmotic final cell volume (V'/V_0) was 0.85 and relative osmotically inactive volume b/V_0 was 0.30. n = 3–4 in each condition.

shows the evolution of the relative cell volumes with time at different osmolarities. The mean cell volume between 13 and 15 min, relative to V_0, was plotted as a function of the relative inverse osmolarity in Fig. 1B. From these figure, it can be seen that cells behaved as perfect osmometers and the relative cell volume, being a function of the reciprocal osmolarity, was well fitted by Eq. (2).

Cell volume at different NaCl concentrations and constant osmolarity

Recently, we showed that H_20 could be transported against its osmotic gradient in association with monovalent cations (Rouzaire-Dubois and Dubois 2010). Consequently, in the present experiments, the cell volume should be function of the NaCl concentration since KCl was held constant at

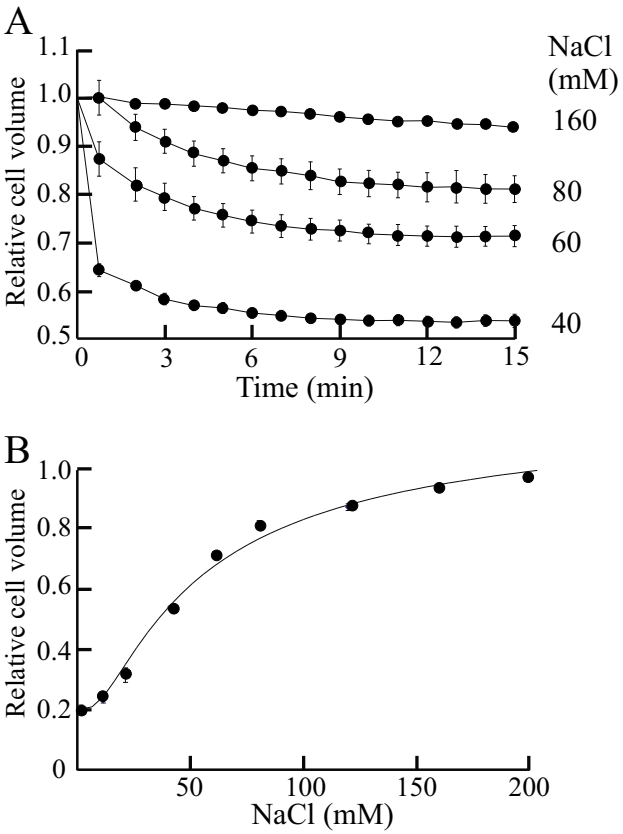

Figure 2. Relative cell volume as a function of the NaCl concentration at constant osmolarity. **A.** Evolution with time of the relative cell volume at different NaCl concentrations. The osmolarity was maintained constant with mannitol. **B.** Mean relative cell volume between 13 and 15 min as a function of the NaCl concentration. The curve was drawn according to Eq. (3) with $V'_{max}/V_0 = 1.00$. The relative NaCl inactive volume (c/V_0) was 0.18; $K_d = 12.25$ mM and $x = 4$. $n = 3–9$ in each condition.

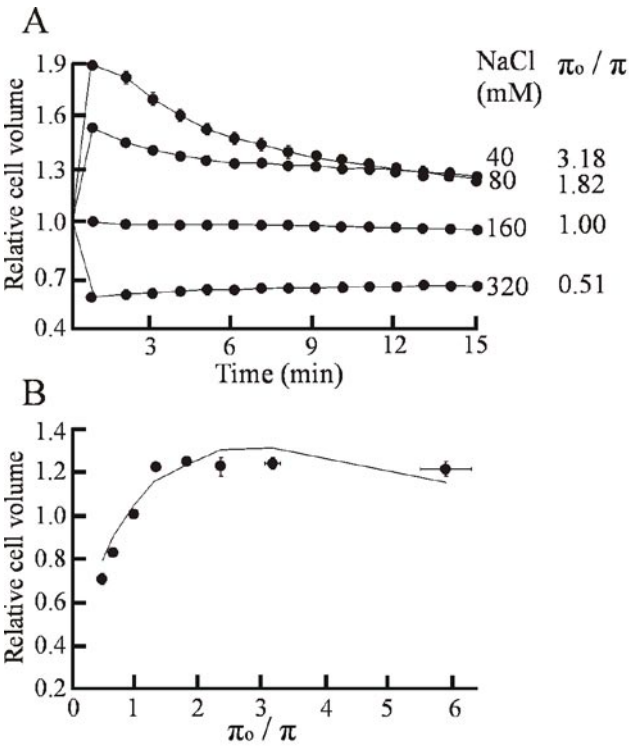

Figure 3. Relative cell volume as a function of osmolarity and NaCl concentration. **A.** Evolution with time of the relative cell volume at different relative osmolarities and NaCl concentrations. **B.** Mean relative cell volume between 13 and 15 min as a function of the relative reciprocal osmolarity. The curve was drawn according to Eq. (4) with $V'_{max}/V_0 = 1$; relative osmotically inactive volume $b/V_0 = 0.15$; $K_d = 25$ mM; $x = 4$; $c/V_0 = 0.15$ and $V'/V_0 = 1.37$. $n = 3–6$ in each condition.

5 mM. In order to confirm this hypothesis, we made experiments with solutions containing different concentrations of NaCl and mannitol so that the osmolarity was constant. Fig. 2A shows the evolution of the relative cell volume with time at different NaCl concentrations. In Fig. 2B the mean cell volume between 13 and 15 min was plotted as a function of the NaCl concentration. From this figure, it can be seen that the relative cell volume was a sigmoid function of the NaCl concentration and could be fitted by the equation:

$$\frac{V}{V_0} = \left[\frac{V'_{max}/V_0}{1 + K_d/[NaCl]}\right]^X + \frac{c}{V_0} \quad (3)$$

where V'_{max} is the maximal cell volume as a function of the NaCl concentration, K_d is the dissociation constant of NaCl with ion channels and c is the NaCl inactive volume. While x can have different values, we fixed it at 4 where the correlation coefficient was superior to 0.99.

Cell volume at different osmolarities and NaCl concentrations

From the above results, it can be predicted that when the osmolarity was modified by changing the NaCl concentration, the relative cell volume as a function of the osmolarity should be described by Eq. (4), which is the product of Eqs. (2) and (3).

$$\frac{V}{V_0} = \left\{\left[\left(\frac{V'-b}{V_0}\right)\frac{\pi_0}{\pi}\right] + \frac{b}{V_0}\right\}\left\{\left[\frac{V'_{max}/V_0}{1+K_d/[NaCl]}\right]^X + \frac{c}{V_0}\right\} \quad (4)$$

Fig. 3A shows the evolution of the relative cell volume with time at different osmolarities and NaCl concentrations. In Fig. 3B, the mean cell volume between 13 and 15 min was plotted as a function of the reciprocal osmolarity. From this figure, it can be seen that the change in cell volume was well described by Eq. (4). In other words, the cells behaved as almost perfect osmometer in hyperosmolarity and hypernatriemia whereas the cell volume saturated in hyposmolarity and hyponatriemia.

Discussion

It is generally accepted that the cell volume changes with osmolarity according to the linear Boyle-van't Hoff law between the relative cell volume and the reciprocal relative osmolarity (Eq. 1). However in different cell types, deviations from this law have often been observed. We previously showed that the cell volume was not only dependent on the osmolarity but also on the NaCl concentration (Rouzaire-Dubois et al. 1999, 2009; Rouzaire-Dubois and Dubois 2010). In the present work, we studied the change in steady-state volume of rat glioma cells under three different conditions, namely: at different osmolarities and constant NaCl concentration; at different NaCl concentrations and constant osmolarity and at different osmolarities and NaCl concentrations. We showed that the relative cell volume was dependent on both the osmolarity and the NaCl concentration and we developed a new equation that takes into account these two parameters. To justify this new equation, we proposed that H_2O is transported downhill through aquaporins, likely AQP 1 in the present model (Rouzaire-Dubois et al. 2009), according to its osmotic gradient and uphill in association with electrolytes through non selective ion channels (Rouzaire-Dubois and Dubois 2010). The relative cell volume variations in Fig. 2B and 3B are well described by Eqs. (3) and (4) with $x = 4$. This suggests that at least four molecules of water are non-cooperatively transported with one molecule NaCl. Another interpretation is that in anisotonic or anisonatriemic conditions, the flux of electrolytes induces changes in intracellular tonicity and osmotically obliged water fluxes. At present, it is impossible to distinguish between these two possibilities. However, it seems unlikely that in hyponatriemia and isotonicity, the cell volume decrease (maximum of 80%, Fig. 2B) was only due to a passive efflux of water. Whatever the interpretation proposed, the cell volume is dependent on both osmolarity and NaCl concentration and deviates from a perfect osmometer in low NaCl concentration. While the observations reported here were obtained in non-physiological conditions, they may have implications in cases of hyponatriemia or stroke.

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