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# Isovolumetric Regulation in Mammal Cells: Role of Taurine

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## 1. Introduction

The ability to regulate cell volume is an ancient conserved trait present in essentially all species through evolution. The maintenance of a constant cell volume is a homeostatic imperative in animal cells. Changes in cell water content affecting the concentration of intracellular messenger molecules impair the complex signaling network, crucial for cell functioning and intercellular communication. Although the renal homeostatic mechanisms exert a precise control of extracellular fluid osmolarity, this is challenged in a variety of pathological situations. The intracellular volume constancy is continuously compromised by the generation of local and transient osmotic microgradients, associated with nutrients uptake, secretion, cytoskeleton remodeling and transynaptic ionic gradients.<sup>1</sup>

Cell volume disturbances have particularly dramatic consequences in the brain. The limits to expansion imposed by the rigid skull give narrow margins for the buffering of intracranial volume changes. As expansion occurs, the constraining of small vessels generates episodes of anoxia, ischemia, excitotoxicity, and neuronal death. In extreme conditions, caudal herniation of the brain parenchyma through the foramen magnum affects brain stem nuclei resulting in death by respiratory and cardiac arrest.

Brain cell swelling occurs either by decreases in external osmolarity as in hyponatremia or by changes in ion redistribution in isosmotic conditions, termed cytotoxic edema. Cells exposed to hyposmotic conditions first swell and then exhibit a volume regulatory response, accomplished by the extrusion of intracellular osmotic solutes, essentially the inorganic osmolytes  $K^+$  and  $Cl^-$ , as well as organic osmolytes such as amino acids, polyamines, and polyalcohols. This is an active regulatory process (termed regulatory volume

decrease, RVD) which occurs in spite of the persistence of the hyposmotic condition. In most studies, RVD is investigated by exposure of cells to abrupt and large decreases in external osmolarity. Although this condition has been useful to characterize and amplify the events occurring during RVD, these drastic changes in external osmolarity occur only seldom, even in pathological situations.

An experimental paradigm developed by Lohr and Grantham<sup>2</sup> in renal cells is that of exposure of cells to small and gradual changes in osmolarity. Under these conditions, cell volume remains constant even after some time of exposure to the osmotic gradient, when the external osmolarity has been considerably reduced. This response was named "isovolumetric regulation" (IVR). We have characterized this phenomenon in various brain cell preparations, including hippocampal slices, cerebellar granule neurons in culture, and C6 glioma cells. That taurine plays an important role in IVR was demonstrated in all cases.

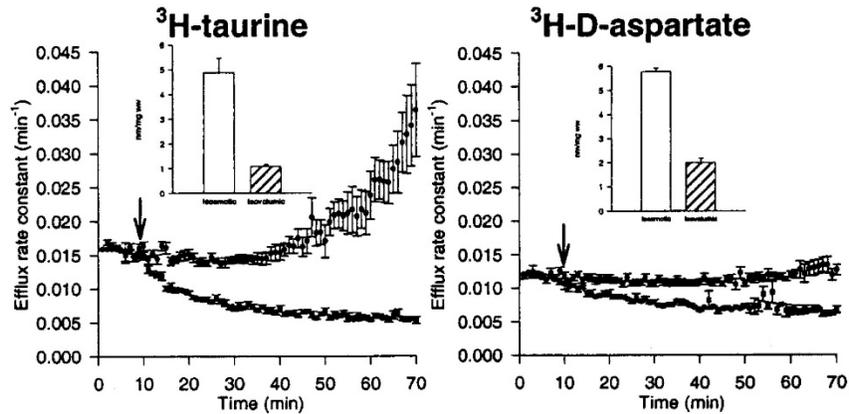
### ***1.1. Mechanism of Isovolumetric Regulation***

The volume constancy observed in cells exposed to an osmotic gradient may reflect either the absence of swelling in these conditions or the activation of mechanisms for volume adjustment. The study by Lohr and Grantham supported the last conclusion, since cells exposed to gradual decreases in osmolarity, which present IVR, shrink when returned to an isosmotic medium, indicative of loss of intracellular osmolytes. IVR in renal cells is blocked by ouabain, quinine, and barium, indicating the involvement of K<sup>+</sup> as an osmolyte in this process.<sup>3</sup> Also in A6 cells, a 70% loss of intracellular K<sup>+</sup> was found after exposure to an osmotic gradient.<sup>4</sup> No other potential osmolyte was examined in those studies.

Subsequently, Souza<sup>5</sup> studied IVR in cultured chick embryo cardiomyocytes. In contrast to renal cells, in cardiomyocytes only partial IVR was observed, since cells started swelling when the external osmolarity had dropped by approximately 15%. At the end of the experiment, a 35% decrease in K<sup>+</sup> and a 22% decrease in taurine cell content were observed. IVR seems not to be a general feature of all cells. Trout erythrocytes were unable to regulate volume in the presence of an osmotic change as low as -0.7 mOsm/min. Neither K<sup>+</sup> nor taurine fluxes were activated in these cells which explains the absence of the volume regulatory response.<sup>6</sup>

Hippocampal slices<sup>7</sup> exposed to an osmotic gradient of -2.5 mOsm/min, are also able to maintain a constant volume. In this preparation, no significant change in K<sup>+</sup> was found during IVR. In contrast, a marked decrease in the concentrations of amino acids of approximately 60% occurred during IVR. The marked reduction of endogenous taurine and glutamate levels of 80% and 65%, respectively, emphasize their contribution to IVR (fig. 1). Radiolabeled amino acids were used to examine the time course of taurine, glutamate, and GABA release in response to the osmotic gradient. [<sup>3</sup>H] Taurine release is observed almost immediately after the reduction in osmolarity, with a threshold of -5 mOsm, while that of glutamate (traced by <sup>3</sup>H-aspartate) and [<sup>3</sup>H] GABA were -20 mOsm. Also the efflux rate of taurine is notably higher, with a maximal increase of 7-fold over the isosmotic efflux while those of glutamate and GABA were only 2- and 3-fold higher, respectively. Interestingly, a similar early release of taurine has been observed *in vivo* in rat brain in response to hyponatremia.<sup>8</sup> All together, these results support the contribution of amino acids to IVR. The apparent lack of contribution of K<sup>+</sup> to the regulatory process in hippocampal slices is

somewhat surprising since this ion is actively participating in IVR in renal cells, A6 cells, and cardiomyocytes. It is possible that in an integrated preparation such as the hippocampal slice, an active buffering mechanism of extracellular  $K^+$  masks the osmosensitive release occurring gradually during IVR. Due to the key role of  $K^+$  in the control of neuronal excitability, its extracellular levels have to be strictly controlled.

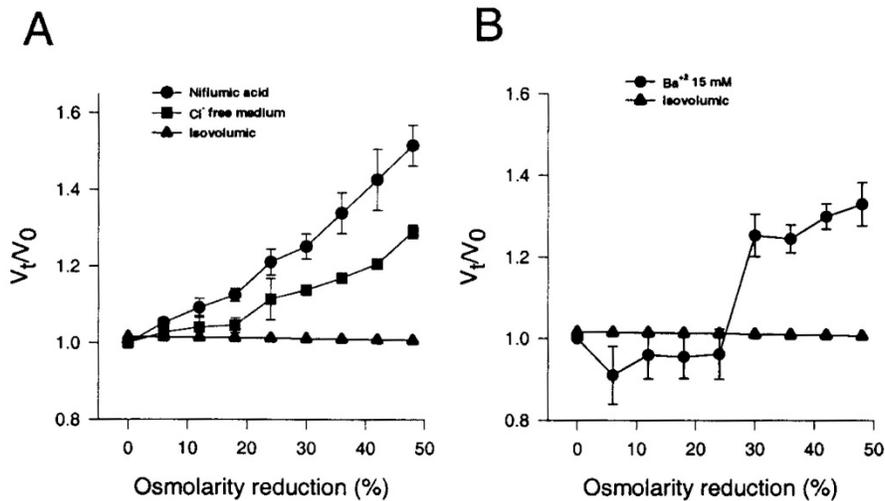


**Figure 1.** Efflux of  $^3\text{H}$ -taurine and  $^3\text{H}$ -D-aspartate from hippocampal slices exposed (arrow) to medium of decreased osmolarity ( $\bullet$ ) at a rate of  $2.5 \text{ mOsm min}^{-1}$ . Controls ( $\blacksquare$ ) were superfused with isosmotic medium. Data are expressed as efflux rate constants ( $\text{min}^{-1}$ ) and are means  $\pm$  SEM ( $n = 8-10$ ). The insets show the decrease of endogenous levels  $\pm$  SEM after expose to isosmotic medium (empty bars) or to the osmotic gradient conditions (dashed bars).

Full IVR occurs in cultured cerebellar granule neurons exposed to gradual changes in external osmolarity of  $-1.8 \text{ mOsm/min}$ .<sup>9</sup> No cell volume increase is observed even when the external osmolarity has dropped by 50%. The contribution of  $K^+$ ,  $\text{Cl}^-$  to IVR and of some amino acids has been examined in this preparation. As in hippocampal slices, taurine exhibited the earliest efflux threshold, of  $-2\% \text{ mosm}$ . Other amino acids are also released in response to the osmotic gradient, with thresholds of  $-19$  and  $-30 \text{ mOsm}$  for glutamate and glycine, respectively.  $\text{Cl}^-$  (followed as  $^{125}\text{I}$ ) and  $K^+$  (as  $^{86}\text{Rb}$ ) efflux showed a release threshold of  $-25$  and  $-29 \text{ mOsm}$ , respectively. These results suggest a contribution by amino acids in the early phases of IVR, while ions have a contribution only late in the process.

To evaluate the active contribution of these different osmolytes, suppression of their fluxes by several experimental maneuvers was attempted. The rationale for these experiments is that a reduction in osmolyte extrusion may result in an increase in cell volume, corresponding to the fraction impaired by the blockade of the osmolyte involved.  $K^+$  efflux is blocked by  $\text{Ba}^{+2}$  and in the presence of this agent, cell swelling occurs at external osmolarity reduced by 24%, coincident with the  $^{86}\text{Rb}$  release threshold (fig. 2B). Similarly cell treatment with solutions in which all  $\text{Cl}^-$  was replaced by gluconate, lead to cell swelling when the external osmolarity is reduced by  $-24\%$ , progressively increasing to reach a maximum of 29% when the external osmolarity is  $-50\%$ . Niflumic acid is a blocker of both

taurine and  $\text{Cl}^-$  efflux during IVR. In the presence of this compound, swelling is observed from the first minutes of exposure to the osmotic gradient, and this increase continues to attain a maximal of 50%. This observation indicates that the early phase of volume regulation occurs entirely by a niflumic-acid sensitive mechanism (fig. 2A). Comparing cell swelling in a medium without  $\text{Cl}^-$ , which affects only the  $\text{Cl}^-$  contribution but not that of organic osmolytes, and swelling in niflumic acid-containing solutions, gives an estimate of the contribution of organic osmolytes. The differences between the two curves suggest an early, continuous, and substantial contribution of these types of osmolytes throughout the IVR process. The particular features of taurine efflux, being more sensitive and more robust than that of other amino acids, underline its predominant role in volume regulation under IVR conditions.



**Figure 2.** Impairment of IVR by niflumic acid,  $\text{Ba}^{2+}$ , and  $\text{Cl}^-$ -free medium. A. Cell volume measured in cells exposed to the osmotic gradient ( $\blacktriangle$ ), in the presence of  $600 \mu\text{M}$  niflumic acid ( $\bullet$ ), or  $\text{Cl}^-$ -free medium ( $\text{Cl}^-$  replaced by gluconates) ( $\blacksquare$ ). In B, cell volume in the presence of  $15 \text{ mM}$   $\text{Ba}^{2+}$  ( $\bullet$ ). Data are means  $\pm$  SEM of 3–4 separate experiments.

Preliminary studies in C6 cells further support this notion, although mainly by negative results. In these cells, the osmotic gradient effectively elicits  $\text{Cl}^-$  and  $\text{K}^+$  currents, which, however, are not sufficient to maintain a constant cell volume. Interestingly, the osmotic gradient is unable to evoke taurine efflux. Not even sudden changes in osmolarity of  $-30\%$  evoke any detectable efflux of taurine. Only very large decreases in osmolarity mobilize taurine.

All together, these observations stress the importance of taurine as a volume regulatory element in conditions which, as mentioned before, may better fit the physiological and pathological situations.

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## References

1. Lang, F., Busch, G. L., Ritter, M., Völki, H., Waldegger, S., Gulbins, E., and Häussinger, D. 1998, Functional significance of cell volume regulatory mechanisms. *Physiol. Rev.* 18, 247–306.
2. Lohr, J. W., and Grantham, J. J. 1986, Isovolumetric regulation of isolated S<sub>2</sub> proximal tubules in anisotonic media. *J. Clin. Invest.* 78, 1165–1172.
3. Lohr, J. W. 1990, Isovolumetric regulation of renal proximal tubules in hypotonic medium. *Renal Physiol. Biochem.* 13, 233–240.
4. Van Driessche, W., De Smet, P., Li, J., Allen, S., Zizi, M., and Mountian, I. 1997, Isovolumetric regulation in a distal nephron cell line (A6). *Am. J. Physiol.* 272, C1890–C1898.
5. Souza, M. M., Boyle, R. T., and Lieberman, M. 2000, Different physiological mechanisms control isovolumetric regulation and regulatory volume decrease in chick embryocardiomyocytes. *Cell Biol. Int.* 24, 713–721.
6. Godart, H., Ellory J. C., and Motais, R. 1999, Regulatory volume response of erythrocytes exposed to a gradual and slow decrease in medium osmolarity. *Pflugers Arch. Eur. J. Physiol.* 437, 776–779.
7. Franco R., Quesada, O., and Pasantes-Morales, H. 2000, Efflux of osmolyte amino acids during isovolumic regulation in hippocampal slices. *J Neurosci. Res.* 61, 701–11.
8. Solís, J. M., Herranz, A. S., Herraz, O., Lerma, J., and Del Rio, R. M. 1988, Does taurine acts as an osmoregulatory substance in the rat brain? *Neurosci. Lett.* 91: 53–58.
9. Tuz, K., Ordaz, B., Vaca, L., Quesada, O., and Pasantes-Morales, H. 2001, Isovolumetric regulation mechanism in cultured cerebellar granule neurons. *J. Neurochem.* 79: 1–10.