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Isovolumic Regulation in Nervous Tissue: A Novel Mechanism of Cell Volume Regulation

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Introduction

Cell volume regulation is a property present in most animal cell lineages that allows them to recover their original volume after events of swelling or shrinkage. Such events can be caused by changes in external osmolarity or to osmotic gradients generated during normal cell functioning.[4,6] The mechanism of cell volume regulation involves transmembrane fluxes of osmotically active solutes in the necessary direction to counteract the net gain or loss of intracellular water.[9] The process through which cells recover their normal volume after swelling is named Regulatory Volume Decrease (RVD). This consists of the efflux of inorganic osmolytes, such as K^+ and Cl^-, as well as organic compounds such as free amino acids, methyl amines, and polyalcohols. These movements create a new osmotic gradient that leads to water efflux and volume recovery.

The simplest and more often experimental paradigm used in RVD studies consists of sudden exposures of cells/tissues to mild-to-acute hyposmotic media (~20 to 50% hyposmotic) and recording different cell parameters (cell volume, osmolyte release, membrane potential, conductances, etc.) These conditions, however, do not occur under normal, physiological circumstances, where changes in external or internal osmolarity are gradual, as the different homeostatic systems are challenged and activated.[12] Even in pathological situations, such as water intoxication or hyponatremia, the activation and eventual surpassing of the encephalic mechanisms of water and electrolyte control lead to *progressive* osmotic changes in the extracellular brain milieu.[3]

Gradual and continuous changes in external osmolarity (in contrast to the sudden changes usually used) were introduced by Lohr and Grantham[8] to examine volume regulation properties in the S_2 proximal tubules. It was observed that regardless of the progressive reduction in the osmolarity medium *cells do not swell*, provided that the rate of change does not exceed –3.0 mOsmol/min. The adaptive cell response occurred, however, as cells swell immediately after reintroduction of isosmotic medium.[8] The response was described as Isovolumic Regulation due to the lack of change in cell volume. In the present study, we described the occurrence of Isovolumic Regulation in different preparations of nervous tissue, where it is observed, the early activation of taurine and glutamic acid efflux, as well as the relatively late (or absence of) mobilization of K^+.

Results and Discussion

Although the experimental model of large and sudden decreases in osmolarity had rendered valuable information to elucidate some basic mechanisms of cell volume control, such changes probably never occur in the brain under physiological conditions. This is also true during pathological situations such as chronic hyponatremia, water intoxication, or the inappropriate secretion of vasopressin, when the osmolarity changes in the brain interstitial space occurs most likely in a gradual manner, as the osmotic challenge from plasma progressively surpasses the brain homeostatic resistance.[3,12] Thus, the experimental approach of the present work, decreasing gradually and slowly the external osmolarity, could reflect more accurately physiological variations. Under these conditions, figure 1 shows the lack of change in cell volume when hippocampal slices (A) or cultivated neurones (B) are exposed to the osmotic gradient, regardless of the low, final external osmolarity (~150 mOsmol/l or 50% hyposmotic). This constancy in cell volume appears to result from an active process of volume control accomplished by the adjustment of osmolyte intracellular content and its named Isovolumic Regulation (IVR). This is supported by the swelling observed in cells previously exposed to gradual hyposmotic changes and suddenly returned to isosmotic medium.[8]

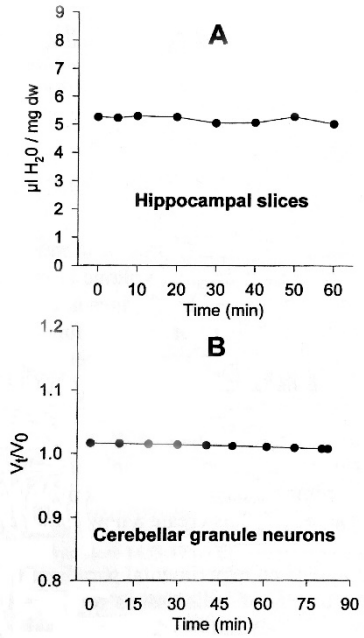

Figure 1. Cell volume changes of hippocampal slices and cerebellar granule neurones exposed to a continuous hyposmotic gradient (300 mOsmol/l ⇒ 150 mOsmol/l). A gradient-generating system was constructed as described by Van Driessche et al.[13] The rate of change in osmolarity was adjusted at –2.5 or –1 .8 mOsmol/min for the experiments with hippocampal slices or cell cultures, respectively. A and B: At time 0 in figures, slices or cerebellar granule neurones were superfused with the osmotic gradient and cell volume was determined at different times. A. Volume changes in hippocampal slices were indirectly estimated by quantification of tissue water content corrected by the interstitial space measured by ^{14}C-inulin distribution. B. Relative cell volume in cerebellar granule neurones was quantified fluorometrically, using calcein-AM as fluorescent dye.[1] Data are means ± SE of 6 (A) or 3 (B) individual experiments.

Figure 2 shows the efflux of ^{3}H-taurine and ^{3}H-D-aspartate from hippocampal slices (A), cerebellar granule neurones (B), or cerebellar astrocytes (C) elicited by a continuous hyposmotic gradient (300 ⇒ 150 mOsmol/l). In these preparations it is observed first, that efflux of both amino acids is activated early during IVR; second, the lower the external osmolarity is, the faster the amino acid efflux, showing no inactivation phase; third, the release of taurine is larger than that of D-aspartate. A similar higher efflux rate for taurine as compared with other osmolytes has been observed in rat brain *in vivo* upon microdialysis perfusion with hyposmotic solutions.[2,11]

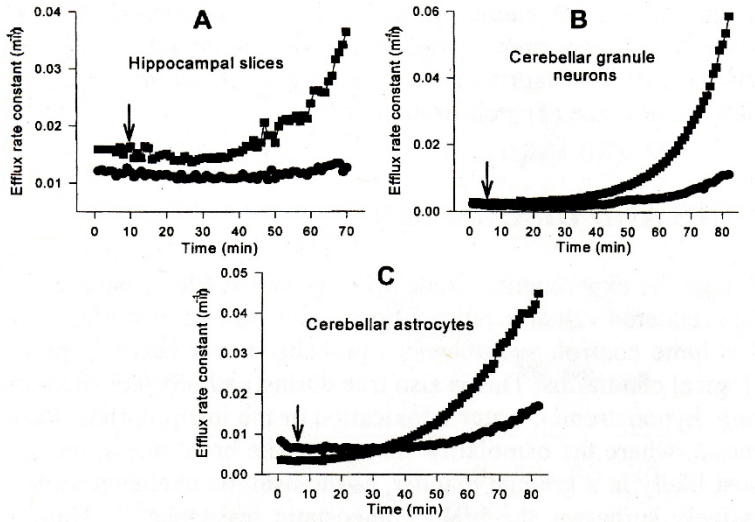

Figure 2. Amino acid efflux from hippocampal slices, cerebellar granule neurones, and cerebellar astrocytes elicited by a continuous hyposmotic gradient. Preloaded tissues were initially superfused with isosmotic medium (6–10 min) and then (at arrow) exposed to the osmotic gradient (300 ⇒ 150 mOsmol/l). (■): ^{3}H-taurine; (●): ^{3}H-D-aspartate in all figures. Data are expressed as efflux rate constants and are means ± SE of 6 (A), 8 (B), or 2 (C) individual experiments.

In the present study, the release of taurine and D-aspartate during IVR were resolved into three first-order velocity components by fitting linear regressions to different segments of the efflux curves. Table 1 shows the derived kinetic constants for hippocampal slices and cerebellar granule neurones, along with the corresponding external osmolarity ranges. The osmotic intervals for each of the release components of taurine and D-aspartate are remarkably similar, suggesting a common efflux pathway.

Table 1. Kinetic constants of ^{3}H-taurine and ^{3}H-D-aspartate fluxes from cerebellar granule neurones and hippocampal slices elicited by a continuous hyposmotic gradient

Cerebellar Granule Neurones	k_1(x10^{-5})	k_2(x10^{-5})	k_3(x10^{-5})
External osmolarity range (mOsmol/l)	300 → 249	248 → 203	202 → 156
^{3}H-D-Aspartate	N.A.	10.37 ± 0.53	37.17± 1.26
^{3}H-Taurine	5.29 ± 0.33	34.97 ± 1.59	213.38 ± 10.12
Hippocampal Slices	k_1(xl0^{-5})	k_2(x10^{-5})	k_3(xl0^{-5})
External osmolarity range (mOsmol/l)	300 → 250	249 → 178	175 → 150
^{3}H-D-Aspartate	–2.322 ± 0.111	2.070 ± 0.219	7.138 ± 1.070
^{3}H-Taurine	–4.066 ± 0.542	8.968 ± 0.502	34.829 ± 3.733

The efflux of ^{3}H-taurine and ^{3}H-aspartate under IVR conditions were kinetically analyzed adjusting the experimental data of figure 1 to lineal regressions in different segments of the curves, as indicated (fractions). Values are the slopes of the adjusted averaged points ± S.E. (n= 4–8) N.A.: Not adjusted

Moreover, such intervals are similar also between preparations, i.e. k_1 describe taurine and D-aspartate fluxes during the same osmotic range in hippocampal slices and granule neurones. This suggests that the efflux mechanisms present in both preparations have similar osmotic sensitivities. The magnitude of the rate release for each amino acid, however, is notably different. In the hippocampal slices, k_1 for taurine is about half of that of D-aspartate (table 1). When the amino acids efflux is described by k_2 and k_3, velocity of taurine efflux is 4 times higher than that of D-aspartate. Similar differences are observed in cerebellar granule neurones: k_2 and k_3 for taurine efflux are about 3.5 and 5.7 times higher than those of D-aspartate. These differences could be due to distinct permeability coefficients through the suggested common pathway, or/and to different availabilities of the intracellular pools. Brain glutamate is extremely active and deeply involved in synaptic transmission and thus is sequestered into vesicles and other metabolic compartments. Taurine in contrast, is essentially an inert compound, not contributing to protein synthesis nor involved in any metabolic reaction, and is found essentially free in the cytosol.[5] All these results suggest a predominant role for taurine in volume regulation in brain.

Potassium is an important osmolyte due to its high intracellular content. An osmosensitive release of K^+ has been consistently described in cells showing regulatory volume decrease (RVD).[6,9] In cerebellar astrocytes, it has been shown that K^+ efflux is the rate-limiting factor in an ongoing RVD process.[10] In isovolumic conditions, an osmosensitive K^+ outflow has been clearly shown in renal cells. In the distal nephron cell line A6, K^+ efflux is activated with a threshold[13] of 210 mOsmol/l, while in renal proximal tubules K^+ content decreased after superfusion with an osmotic gradient.[7] Figure 3 shows the release of K^+ (traced with ^{86}Rb) during IVR, from hippocampal slices (A) and cerebellar granule neurones (B). In this last preparation, K^+ outflow initially follows a k_1 equal to $4.98 \pm 0.36 \times 10^{-5}$ until the external osmolarity has decreased 90 mOsmol (≈ 30% hyposmotic). This value is in the same range as that measured in isosmotic conditions ($3.48 \pm 0.15 \times 10^{-5}$). When the external osmolarity has decreased ≈ 65 mOsmol, K^+ efflux is activated and its movement follows a second k, with a value of $39.97 \pm 1.04 \times 10^{-5}$ for the rest of the experiment. In contrast, ^{86}Rb outflow from hippocampal slices *does not change* during IVR, and its efflux runs parallel with that observed under isosmotic conditions. This is an unexpected result, since as previously mentioned, K^+ is a key osmolyte in essentially all cell types. The difference in K^+ efflux during RVD and IVR may be due to the involvement, in each case, of different mechanisms of release. In renal proximal tubules, the Na^+-K^+ ATPase seems implicated in IVR[7] but not in RVD. Also, these two processes differ in A6 cells[13] . In addition, unlike in cells in culture, in the hippocampal slices which have an intact cytoarchitecture, buffering of extracellular K^+ by the efficient mechanisms known to exist in brain tissue, could mask an osmosensitive release occurring gradually as during IVR. Due to the key role played by K^+ in nervous excitability, its extracellular levels in brain have to be kept under strict control. Clearly, studies on the occurrence and features of IVR in different cell types are essential for a better understanding of the physiological significance of this mechanism of volume regulation.

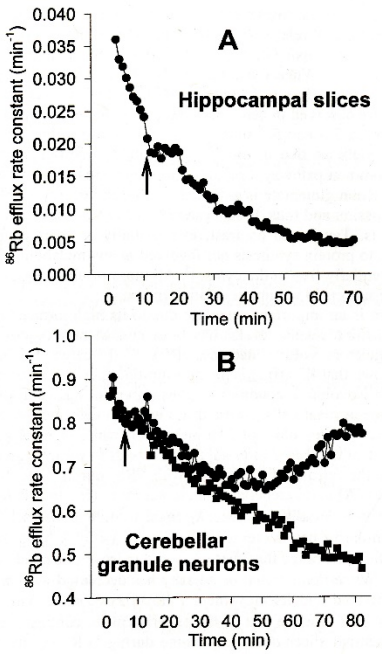

Figure 3. ^{86}Rb efflux from hippocampal slices and cerebellar granule neurones elicited by a continuous hyposmotic gradient. Preloaded tissues were initially superfused with isosmotic medium (6–10 min) and then (at arrow) exposed to the osmotic gradient (300 mOsmol/l ⇒ 150 mOsmol/l). A and B (●): Hyposmotic gradient; B (■): Isosmotic medium. Data are expressed as efflux rate constants and are means ± SE of 6 (A) or 8 (B) individual experiments.

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