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Efflux of Osmolyte Amino Acids during Isovolumic Regulation in Hippocampal Slices

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Abstract

The efflux of potassium (K^+) and amino acids from hippocampal slices was measured after sudden exposure to 10% (270 mOsm), 25% (225 mOsm) or 50% (150 mOsm) hyposmotic solutions or after gradual decrease (22.5 mOsm/min) in external osmolarity. In slices suddenly exposed to 50% hyposmotic solutions, swelling was followed by partial (74%) cell volume recovery, suggesting regulatory volume decrease (RVD). With gradual hyposmotic changes, no increase in cell water content was observed even when the solution at the end of the experiment was 50% hyposmotic, showing the occurrence of isovolumic regulation (IVR). The gradual decrease in osmolarity elicited the efflux of 3H -taurine with a threshold at -5 mOsm and D- 3H aspartate (as marker for glutamate) and at -20 mOsm for 3H GABA. The efflux rate of 3H taurine was always notably higher than those of 3H GABA and D- 3H aspartate, with a maximal increase over the isosmotic efflux of about 7-fold for 3H taurine and 3- and 2-fold for 3H GABA and D- 3H aspartate, respectively. The amino acid content in slices exposed to 50% hyposmotic solutions (abrupt change) during 20 min decreased by 50.6% and 62.6% (gradual change). Taurine and glutamate showed the largest decrease. An enhancement in ^{86}Rb efflux and a corresponding decrease in K^+ tissue content was seen in association with RVD but not with IVR. These results demonstrate the contribution of amino acids to IVR and indicate their involvement in this mechanism of cell volume control.

Keywords: taurine, cell swelling, K efflux, regulatory volume decrease, hyposmolarity

Cell volume control is maintained by the transmembrane fluxes of osmotically active solutes in the necessary direction to counteract water movements caused by changes in external osmolarity or by osmotic gradients originated during the normal cell functioning (Hoffmann and Dunham, 1995; Häussinger, 1996; Lang et al., 1998). Osmolyte translocation subsequent to hyposmotic cell swelling has been extensively investigated in a variety of preparations (Pasantés-Morales, 1996; Strange et al., 1996; Kirk, 1997; Nilius et al., 1997). Most of these studies refer to osmolyte fluxes activated in response to abrupt and large decreases in external osmolarity whereas less is known about the osmolyte movements after isosmotic swelling or after small and gradual reductions in external osmolarity, an approach more closely resembling pathophysiological situations (Kimelberg and Ransom, 1986; McManus and Churchwell, 1994; Fraser and Arieff, 1997). An early study in renal proximal tubules (Lohr and Grantham, 1986) demonstrated that cells maintain their normal size within a large range of external osmolarities if the change occurs slowly and gradually. This phenomenon has been named isovolumetric or isovolumic regulation (IVR). This term may not mean that the cell volume is not increasing but rather that it is maintained by microadjustments accomplished by the efflux of intracellular osmolytes. This is suggested by the fact that cells gradually exposed to increasingly hyposmotic external solutions, shrink when suddenly returned to isosmotic conditions (Lohr and Grantham, 1986). Information about the osmolytes involved in this process and their mechanisms of translocation is scarce. In the distal nephron cell line (A6), IVR leads to an efflux of K^+ but no other osmolytes have been identified yet (Van Driessche et al., 1997). In the present work, we addressed to this point in hippocampal slices measuring the efflux of K^+ and some amino acids known to play a role as osmolytes, i.e., glutamate, GABA and taurine, in response to gradual and continuous reductions (2.5 mOsm/min) in external osmolarity. The threshold and rate of release of the different osmolytes was compared to that elicited by acute, large changes in osmolarity. To our knowledge, this is the first time that changes in osmolyte amino acids during IVR have been reported.

Materials and Methods

Materials

5-Nitro-[3-phenylpropylamino]benzoic acid (NPPB) was from Research Biochemicals Inc. (Natick, MA); 1,9, dideoxyforskolin (DDF) and niflumic acid were from Sigma Co. (St. Louis, MO). All radiolabeled compounds were from New England Nuclear (Boston, MA).

Hippocampal Slices

Male adult Wistar rats (200–250 g body weight) were used throughout the study. Animals were killed by decapitation and brains rapidly removed and submerged into cold Krebs-Hepes medium that contained (in mM): 135 NaCl, 1.0 CaCl₂, 1.17 MgCl₂, 1.7 KH₂PO₄, 5 KCl, 5 dextrose, and 10 HEPES (300 mOsm/l, isosmotic, pH 7.4). Hippocampus from both hemispheres were dissected and transverse slices (400 μ m thick) obtained with a McIlwain tissue chopper. Slices were immediately submerged into isosmotic medium continuously bubbled with O₂ and kept at room temperature at least for half an hour before the assays.

Experimental Solutions

Hyposmotic solutions [270 mOsm/l (10%); 225 mOsm/l (25%); 150 mOsm/l (50%)], were prepared by reducing the concentration of NaCl accordingly. Final osmolarities were verified with a freezing-point osmometer (Osmette A, Precision Systems, Inc., Natick, MA). To obtain continuous and gradual changes in osmolarity, a gradient-generating system was constructed as described in detail by Van Driessche et al. (1997). Briefly, the system consisted of two identical glass cylinders interconnected at their bases by a tube with an interrupting valve. The first container was filled with isosmotic medium and the second with the same volume of 50% hyposmotic medium. Media were kept at 37°C placing the cylinders on a temperature-controlled hot plate with stirring. Perfusion medium was pulled from the first container with a peristaltic pump, producing that 50% hyposmotic medium begin to enter this cylinder, mixing gradually and continuously with the isosmotic medium. In this way, an osmotic gradient is generated with a rate of change of -2.5 mOsm/min. At the end of the experiment (60 min later) the superfusion medium reached 150 mOsm/l (50% hyposmotic).

Tissue Water Content

Changes in hippocampal slice volume were indirectly estimated by quantification of the tissue water content and [¹⁴C]inulin distribution. For measuring tissue water content, previously weighted slices kept in isosmotic media at 37°C were either, incubated in media of decreased osmolarity during the time indicated at each experiment or superfused with an increasingly hyposmotic medium (osmotic gradient), until the external osmolarity reached 150 mOsm (50% hyposmotic). At the end of experiments, slices were recovered, blotted, dried in oven for 24 hr at 90°C, and re-weighted to obtain the dry weight. Results are expressed as μ l of water per mg of tissue dry weight. To estimate the changes of the intracellular volume the distribution of ¹⁴C-inulin was assessed after the procedure of Fishman et al. (1977). Slices were incubated with 18.5 MBq/ml of [¹⁴C]inulin during 60 min. At the end of this period, slices were blotted and transferred to the experimental (isosmotic or hyposmotic) media containing the same concentration of [¹⁴C]inulin. At the time indicated in each experiment, the slices were removed from the medium, blotted and the retained radioactivity and radioactivity in the medium, measured in a scintillation spectrometer. Extracellular space in rat hippocampal slices was estimated as 17% of total water content in isosmotic conditions, while the percentage change of intracellular volume was calculated from the [¹⁴C]inulin data and expressed as μ l of water/mg dry weight.

Labeled Amino Acids and ⁸⁶Rb Efflux

Hippocampal slices were incubated in isosmotic medium with the labeled amino acids, [³H]taurine (60 min, 55.5 MBq/ml), [³H]GABA or D-[³H]aspartate (as a metabolically inert analogue of glutamate) (30 min, 37 MBq/ml), or with ⁸⁶Rb as a tracer for K⁺ (30 min, 74 MBq/ml). After the loading period, slices were transferred into perfusion chambers (0.4 ml vol) and washed by perfusion with warmed (37°C) isosmotic medium at a rate of 1 ml/min during 15 min. From hereafter, samples were collected every minute and after 5–10 min of basal release, the osmolarity of the perfusion medium was suddenly or gradually decreased as follows. In the first case, the perfusion medium (isosmotic) was switched to one

of reduced osmolarity (either 10, 25, or 50 % hyposmotic) and samples were collected during 25 min. In the second case, slices were superfused with the osmotic gradient and samples were collected every min up to 60 min. When the effect of Cl⁻ channel blockers was examined, drugs were preincubated during 15 min and were present through the whole experiment. Controls contained the corresponding vehicle. The release of labeled amino acids and that of ⁸⁶Rb was expressed as efflux rate constants, i.e., the radioactivity released at any given time as percent of total radioactivity present in the cells at that time.

Measurement of Amino Acid and K⁺ Content in Slices

After perfusing tissue with the experimental solutions indicated, individual slices were homogenized in 500 µl of water. Amino acids were extracted with 70% ethanol, mixed with *o*-phthalaldehyde and quantified by standard reverse phase HPLC. Endogenous K⁺ content was measured in slices previously exposed to one of the experimental paradigms. Slices were digested in boiling HCl (0.1 M, 30 min), then filtered and the K⁺ content determined by atomic absorption spectrometry.

Results

Changes in Water Content

Figure 1A shows the water content of hippocampal slices exposed to 10%, 25%, and 50% hyposmotic solutions during 5 min. An increase in water content paralleled the decrease in external osmolarity. As compared to the isosmotic condition, the water content increased by 22.1, 41.6, and 63.2% in 10%, 25%, and 50% hyposmotic solutions, respectively. In all these experiments, results are expressed as µl of water per mg of dry weight. The interstitial space assessed by [¹⁴C]inulin retention (17%) was subtracted in all cases to obtain the net change in intracellular water space. To examine the ability of cell volume recovery in the slices, the change in intracellular water content was followed at different times after exposure to 50% hyposmotic medium (fig. 1B). Two min after the stimulus, cell volume has increased 58% above cell volume in isosmotic medium and remained unchanged up to 5 min. Thereafter, cell volume progressively decreased and after 20 min, cells have recovered 74.5% of their original volume (fig. 1B), suggesting regulatory volume decrease. Cell volume recovery was not completed within 30 min of exposure (not shown), but returning the slices to isosmotic medium (fig. 1B) rapidly attained the original volume. When the change in external osmolarity was slow and gradual (2.5 mOsm/min), swelling did not occur within the 60 min of exposure to the osmotic gradient, despite the marked decrease in external osmolarity, which at that time has been reduced to 150 mOsm (fig. 1C). These results indicate the occurrence of IVR in hippocampal slices.

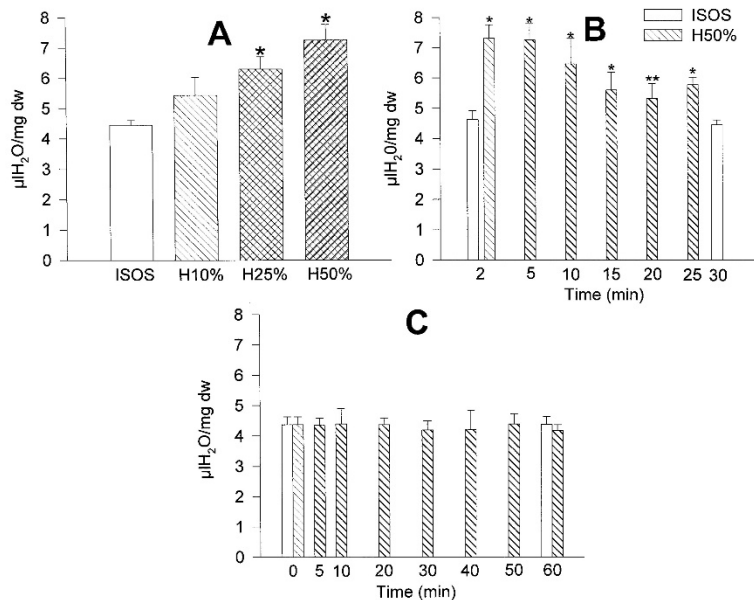


Figure 1. Changes in water content of hippocampal slices suddenly or gradually exposed to hyposmotic media. (A) After a 10 min period in isosmotic medium, hippocampal slices were exposed to solutions of reduced osmolarity (10%, 25%, or 50% hyposmotic) during 5 min. Control slices were kept in isosmotic medium (isos). (B) Slice water content was measured after 2 min incubation in isosmotic medium (empty bar at left), after exposure to 50% hyposmotic medium during the times indicated (dashed bars) or 5 min after return to isosmotic medium (empty bar at right). (C) Slices were superfused with an osmotic gradient (dashed bars) (rate of osmolarity change: 22.5 mOsm/min) and recovered for water content determination at the indicated times. Control slices (empty bars) were superfused 60 min with isosmotic medium and water content determined at the beginning and at the end of the superfusion. Data are expressed as μl of H_2O per mg of tissue dry weight (dw) and are means \pm SE. $n = \text{A: } 8\text{--}10; \text{B: } 4\text{--}8; \text{C: } 4\text{--}8$. * $P < 0.005$, ** $P < 0.05$ as compared to the corresponding isosmotic condition.

Amino Acid Release after Sudden Exposure to Hyposmotic Solutions

Figure 2 shows the release of [^3H]taurine, D-[^3H]aspartate, and [^3H]GABA from hippocampal slices in response to sudden exposure to 50% hyposmotic solutions. The efflux of D-[^3H]aspartate and [^3H]GABA was activated immediately after the stimulus and also rapidly inactivated. The time course of release showed some irregularities that may suggest the occurrence of more than one component in the efflux pattern. The release curves, however, fitted well to a single exponential decay with $r^2 = 0.950$ for D-[^3H]aspartate and 0.976 for [^3H]GABA. During the time of exposure to the hyposmotic medium the release of [^3H]GABA and D-[^3H]aspartate accounted for 52% and 77%, respectively, of the total labeled amino acid incorporated during loading (fig. 2A,B). The efflux of [^3H]taurine elicited by 50% hyposmotic medium increased slowly, reaching the maximal rate release only at 7 min after the stimulus (fig. 2C). More than 80% of the labeled taurine in the preparation was released during the time of exposure to the hyposmotic medium (fig. 2C). Hyposmotic

25% and 10% solutions still evoked a significant increase in the efflux of the three amino acids (fig. 3A–C).

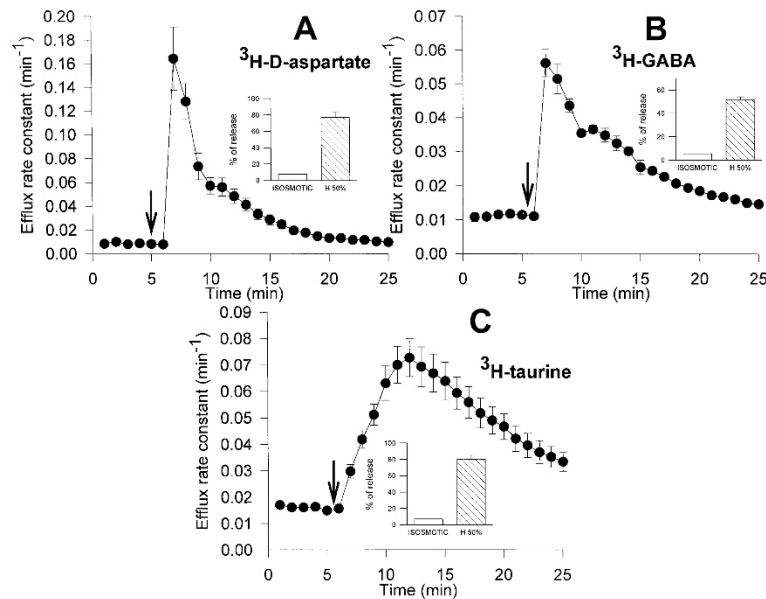


Figure 2. Amino acid release from rat hippocampal slices suddenly exposed to 50% hyposmotic medium. Slices preloaded with D-³H]aspartate (A), [³H]GABA (B), or [³H]-taurine (C) were superfused 5 min with isosmotic medium. At the time pointed by the arrow, the medium was replaced by 50% hyposmotic solution. One min fractions were collected during 20 min. Data are expressed as efflux rate constants (min⁻¹) and are means ± SE (n = 6–10). The insets show the percentage release ± SE of the corresponding labeled amino acid during the time of exposure to the hyposmotic solution (min 6–25).

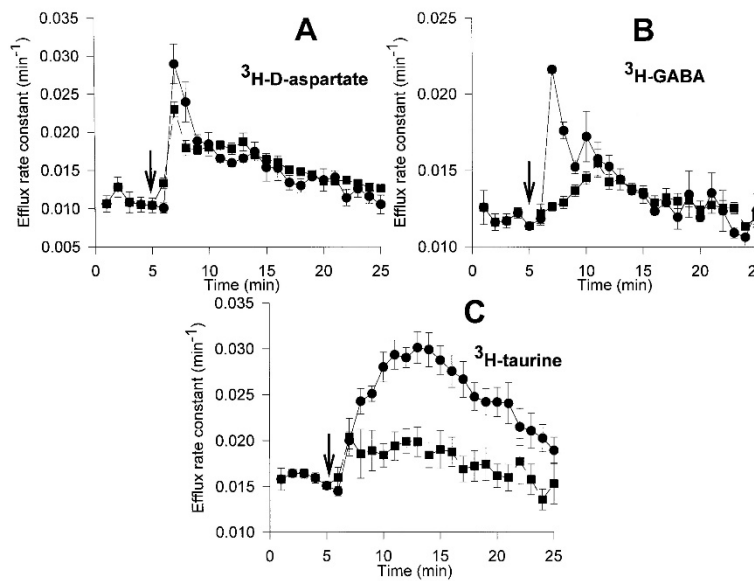


Figure 3. Amino acid release from rat hippocampal slices suddenly exposed to 10% and 25% hyposmotic media. Slices preloaded with D-[^3H]aspartate (A), [^3H]GABA (B), or [^3H]taurine (C) were treated as described in figure 2; 10% (\blacksquare) or 25% (\bullet). Data are expressed as efflux rate constants (min^{-1}) and are means \pm SE ($n = 6-10$). The points in hyposmotic 25% were significantly different from basal release ($P < 0.05$) from 7–16 min for D-[^3H]aspartate, 7–23 for [^3H]GABA, and 6–25 for [^3H]taurine. In 10% hyposmotic medium data were significant from 7–23 for D-[^3H]aspartate, 7–17 for [^3H]GABA, and 10–16 for [^3H]taurine.

To minimize possible effects of reuptake or diffusion through the layers of the slice on the amino acid fluxes, experiments were carried out increasing the perfusion rate to 2 ml per min. This maneuver did not change the time course nor the magnitude of efflux for any of the amino acids as compared to experiments with superfusion rate of 1 ml/min (not shown). Solutions containing the same low NaCl concentration as in 50% hyposmotic solutions but made isosmotic with sucrose did not elicit any efflux of amino acids (not shown).

Amino Acid Release after Exposure to Gradual Decreases in Osmolarity

Figure 4 shows the efflux of [^3H]taurine, [^3H]GABA, and D-[^3H]aspartate from hippocampal slices exposed to medium of osmolarity decreased at a change rate of 2.5 mOsm/min. Slices exposure to the osmotic gradient enhanced the release of [^3H]taurine and D-[^3H]aspartate as early as 2 min after the stimulus, corresponding to a decrease in osmolarity of 5 mOsm. Amino acid release at this time was significantly higher ($P < 0.005$) than in isosmotic medium. The efflux of [^3H]GABA exhibited a higher threshold with significant increases observed at -20 mOsm ($P < 0.005$) (fig. 4). The efflux rate of [^3H]taurine was always notably higher than that of [^3H]GABA and D-[^3H]aspartate, with maximal increases over

the isosmotic efflux of about 7-fold for [^3H]taurine and 3- and 2-fold for [^3H]GABA and D-[^3H]aspartate, respectively.

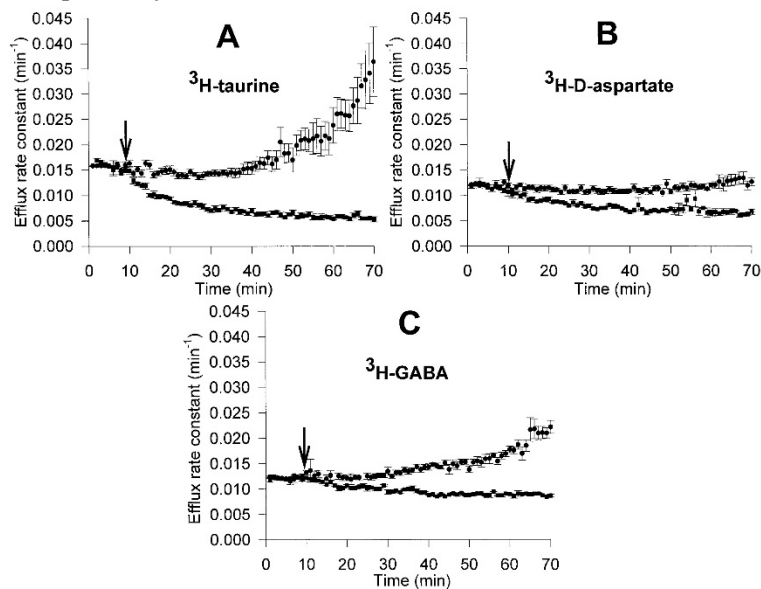


Figure 4. Amino acid release from hippocampal slices exposed to gradual and progressive reductions in external osmolarity. Slices preloaded with [^3H]taurine (A), D-[^3H]aspartate (B), or [^3H]GABA (C), were superfused 10 min with isosmotic medium. At the time pointed by the arrow in (●) the external osmolarity was continuously decreased at a rate of -2.5 mOsm/min until the medium osmolarity reached 150 mOsm (50% hyposmotic). Controls (■) were superfused with isosmotic medium. Data are expressed as efflux rate constants (min^{-1}) and are means \pm SE ($n = 8-10$). Significant differences ($P < 0.005$) between basal release and experimental condition were found from 12th fraction and onward for taurine and D-[^3H]aspartate (A, B), and from the 18th fraction for [^3H]GABA (C).

Effect of Cl^- Channel Blockers

NPPB, DDF, and niflumic acid, known blockers of the osmosensitive Cl^- /amino acid pathway were tested on [^3H]taurine efflux stimulated by sudden or gradual changes in osmolarity. The effect of agents which reduced the volume-sensitive taurine release in cultured brain cells, DDF, NPPB, and niflumic acid (Sánchez-Olea et al., 1996), was tested on the efflux of [^3H]taurine from hippocampal slices during the 20 min of exposure to 50% hyposmotic solution (min 5–25, fig. 5A). DDF (100 μM) did not significantly affected this release, whereas NPPB (100 μM) and niflumic acid (600 μM) decreased it markedly (fig. 5A). The total [^3H]taurine efflux calculated as % release as in figure 2 (inset) was reduced by 58% (NPPB) and 69.6% (niflumic acid). The effect of niflumic acid was then tested on [^3H]taurine efflux during IVR. As shown in figure 5B this agent exhibited the same notable inhibitory action.

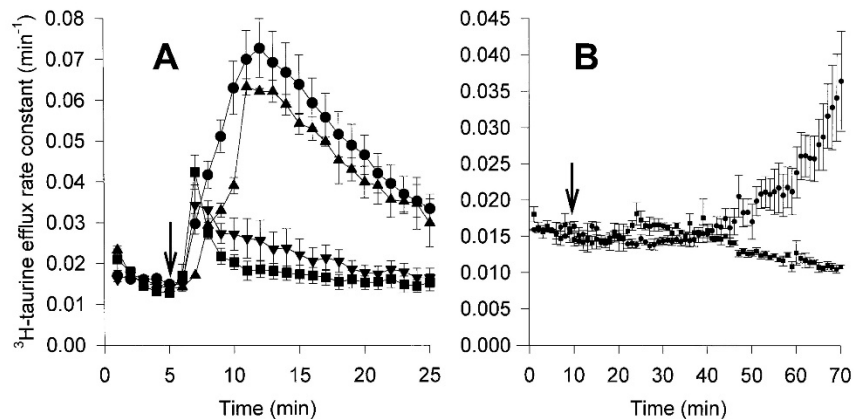


Figure 5. Effect of anion/amino acid channel blockers on the osmosensitive [³H]-taurine efflux from hippocampal slices. (A) Slices preloaded with [³H]-taurine were preincubated during 15 min in isosmotic medium without additions (control: ●), or plus 100 μM DDF (▲), 100 μM NPPB (▼) or 600 μM niflumic acid (■) and then superfused with isosmotic medium and 50% hyposmotic medium (arrow). Blockers were present in all solutions used. Significant differences ($P < 0.005$) were found between control condition and niflumic acid or NPPB, from the 9th fraction. (B) Slices preloaded with [³H]-taurine were exposed to the osmotic gradient in the absence (control, ●) or presence (■) of 600 μM niflumic acid. Control data are the same shown in figure 4A. Data are expressed as efflux rate constants (min⁻¹) and are means \pm SE ($n = 4-8$).

Decrease in Amino Acid Concentration by Hyposmotic Solutions or after IVR

The decrease in the total free amino acid content in the hippocampal slices exposed to 50% hyposmotic solution or to IVR conditions, is shown in figure 6A. A reduction of 50.6% (50% hyposmotic) and 62.6% (IVR) was observed. Amino acids showing the largest decrease in the two conditions were glutamate (52% and 65% in hyposmotic and isovolumic treatment, respectively) and taurine (64% and 78% in the same conditions) (fig. 6B,C). Results are expressed as nmol/mg wet weight. In this condition, some dilution of amino acid levels is expected when the treatment results in a change in cell volume (50% hyposmotic). Because the samples for estimation of amino acid content were taken at min 20, the volume in slices exposed to the hyposmotic medium is still 14.7% higher than in isosmotic medium (fig. 1B). Therefore, a dilution of about 15% is contributing to the observed decrease in the amino acid content under this condition. This does not occur in the IVR paradigm in which there is no volume change.

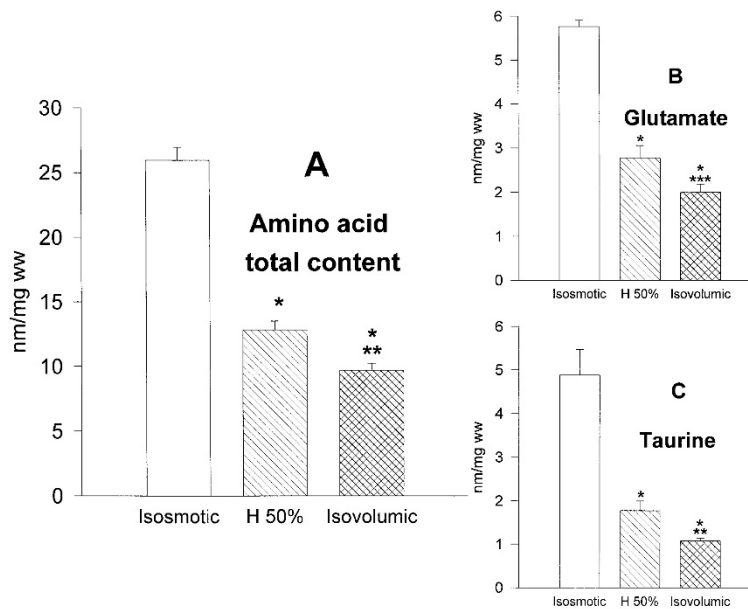


Figure 6. Amino acid content in rat hippocampal slices exposed to sudden or gradual decreases in external osmolarity. Slices were perfused during 10 min with isosmotic medium (empty bars) or with 50% hyposmotic medium (dashed bars) or with an osmotic gradient (isovolumic) for 60 min (crossed bars). At the end of the experiments amino acid content was analyzed by HPLC. (A) Total content. (B) Glutamate content. (C) Taurine content. Data are expressed as nmol/mg wet weight and represent the mean of 8 experiments \pm SE. Asterisks indicate significant differences * $P < 0.001$; ** $P < 0.02$; *** $P < 0.05$ vs. control.

⁸⁶Rb Release and Changes in K⁺ Levels during RVD and IVR

Hippocampal slices loaded with ⁸⁶Rb and suddenly exposed to 10%, 25%, and 50% hyposmotic solutions responded with a fast and transient release of the labeled tracer. The time course release was similar in all solutions, and the rate release increased as the external osmolarity was reduced (fig. 7A). In contrast, exposure to gradual changes in osmolarity did not elicit any significant ⁸⁶Rb efflux (fig. 7B). Figure 7C shows the K⁺ content of slices treated as in ⁸⁶Rb efflux experiments. In 10% and 25% hyposmotic solutions, K⁺ concentration did not significantly decrease. A reduction of about 40% in K⁺ concentration was observed in the slices exposed to 50% hyposmotic solutions. Figure 7D shows the K⁺ content of slices superfused during the indicated times with isosmotic medium (empty bars) or with the gradually diluted medium (dashed bars). Essentially no changes in K⁺ levels were observed through all the superfusion period with isosmotic medium. Also no significant changes were detected after superfusion with the gradually diluted medium (fig. 7D).

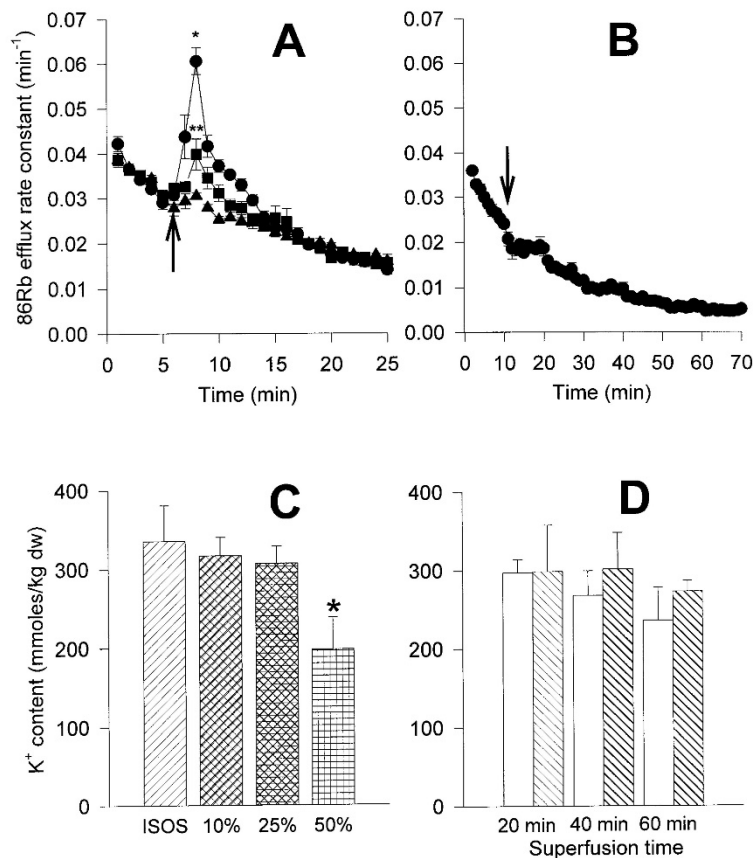


Figure 7. ^{86}Rb efflux and K^+ content in hippocampal slices exposed to sudden or gradual reductions in external osmolarity. (A) Slices preloaded with ^{86}Rb were superfused 10 min with isosmotic medium and then, at the arrow, with solutions of decreased osmolarity: 10% (\blacktriangle), 25% (\blacksquare), or 50% (\bullet), for 20 min. (B) Same as in A, but at min 10 (arrow), the external osmolarity decreased at a rate of 2.5 mOsm/min. Data are expressed as efflux rate constants (min^{-1}) and are means \pm SE ($n = 4-6$). $*P < 0.001$, $**P < 0.05$, as compared with the last basal efflux point. (C) After superfusion with either isosmotic medium or hyposmotic solutions (10%, 25%, or 50%) for 20 min, slices were digested with HCl and the K^+ content measured by atomic absorption spectrometry. (D) Slices superfused with isosmotic medium (empty bars) or exposed to the osmotic gradient (dashed bars) were recovered at different times (20, 40, or 60 min) for determination of K^+ content. Results are expressed as mmol of K^+ /Kg of dry weight. Data are means \pm SE ($n = 3-4$). $*P < 0.05$, as compared to the isosmotic condition.

Discussion

The results of the present work suggest the occurrence of RVD in hippocampal slices after swelling elicited by hyposmotic solutions. This is still a controversial matter in this preparation. In a study measuring changes in cell volume in CA1 neurons, no indication of volume regulation could be found (Andrew et al., 1997) whereas, similar to our results,

another report describes partial cell volume recovery in the whole slice (Chebabo et al., 1995). This discrepancy may be attributable in part, to differences in the cell population in the areas examined or to the magnitude of the hyposmotic challenge. Variations have been reported in the extent of swelling and of volume recovery within the various brain cell types and even within regions of the same cell (Andrew and McVicar, 1994; Chebabo et al., 1995; Baraban et al., 1997). More efficient volume regulation seems to occur associated with stronger osmotic stress (Chebabo et al., 1995).

Amino acid release from brain cells as part of the mechanism of RVD is well documented in a variety of brain tissue preparations, including cultured astrocytes and neurons (Kimmelberg et al., 1990; Pasantes-Morales et al., 1993), brain cortex slices (Law, 1994, 1996), and in brain in vivo (Solís et al., 1988; Estevez et al., 1999). This also occurs in hippocampal slices as shown in the present work. Amino acids are part of the pool of organic osmolytes that contribute to counteract the changes in brain water content during hyponatremia (Lien et al., 1991). The contribution of organic osmolytes to this compensatory response of brain cells has been calculated in about 29%, with the pool of amino acids accounting for approximately 15% (Lien et al., 1991). It is noteworthy that the amino acids preferentially released in response to changes in cell volume, are also neuroactive compounds, and they could consequently affect neuronal excitability. For instance, the efflux of glutamate here observed may contribute to the consistently reported effects of hyposmolarity increasing neuronal firing rate in hippocampal slices (Roper et al., 1992; Huang et al., 1997).

Although the experimental model of sudden and marked decreases in osmolarity had rendered valuable information to elucidate some basic mechanisms of cell volume control, such changes probably never occur in brain under physiological conditions. Even during pathological situations such as chronic hyponatremia, water intoxication or the inappropriate handling of antidiuretic hormone, the osmolarity changes in the interstitial space in brain, occur most likely in a gradual manner as the osmotic challenge from plasma progressively surpasses the brain homeostatic resistances (Trachtman, 1991; McManus and Churchwell, 1994; Fraser and Arief, 1997). Thus, the experimental approach of the present work, having as stimulus a gradual and slow decrease in external osmolarity, reflects more accurately the pathophysiological conditions. This paradigm has been previously used in the distal nephron cell line A6 (Van Driessche et al., 1997) and in renal proximal tubules (Lohr and Grantham, 1986; Lohr, 1990), where it was first described that cell volume remains stable over a broad range of osmolarities, provided that the rate of change is gradual. This constancy in cell volume, named IVR seems to result from an active process of volume control accomplished by the adjustment of osmolyte intracellular content, as evidenced by the shrinkage observed when cells previously exposed to gradual hyposmotic changes are suddenly returned to isosmotic medium (Lohr and Grantham, 1986). The osmolytes involved in IVR are not well known. Studies on renal cells implicate K^+ as an active osmolyte during this process (Lohr, 1990; Van Driessche et al., 1997). In the present work we now showed the contribution of amino acids, preferentially taurine, GABA and glutamate. The efflux of both, taurine and glutamate was responsive to very small changes in osmolarity, of about 5 mOsm. The efflux of taurine, however, was always significantly higher than those of GABA and glutamate, and also taurine exhibited the largest decrease in the intracellular content. Several possible explanations could account for the observed differences

between the three amino acids: (i) a difference in the diffusion through the layers of the slice; (ii) a different permeability coefficient through the osmosensitive pathway; and (iii) the relative availability of amino acid pools to be released upon hyposmolarity. A distinct washout rate due to different diffusional barriers for each amino acid seems unlikely because the amino acid efflux during IVR in our experiments was compared, point to point, with the efflux in isosmotic conditions. This would correct any possible difference related to diffusion. In addition, the amino acid efflux pattern during RVD was opposite to that in IVR, with the release of D-[³H]aspartate being the highest and most rapid and that of [³H]taurine the lowest and most delayed. This is observed even when the superfusion rate in the two paradigms was identical. Finally, in studies on IVR in cultured astrocytes and neurons in monolayer (unpublished results), where the diffusion factor is minimized, still the release of taurine clearly surpasses that of GABA and D-aspartate. From all these results, it is likely that the preferential release of taurine during IVR is related either to higher permeation through the osmosensitive pathway or to more availability of the intracellular taurine pools. A similar early release of taurine as compared to other amino acids has been found in studies in rat brain *in vivo* upon perfusion by microdialysis with media of decreased osmolarity (Solís et al., 1988; Estevez et al., 1999;). All this suggests an important role for taurine in the mechanisms of cell volume control in brain. It is as yet unclear whether the mechanism of amino acid release during IVR and RVD are similar, although the inhibitory effect of niflumic acid points into that direction.

As previously mentioned, K⁺ is clearly implicated in IVR in renal cells (Lohr, 1990; Van Driessche et al., 1997). In A6 cells, K⁺ efflux (traced by radiolabeled ⁸⁶Rb) increases during IVR with a threshold activation at 210 mOsm (about 30% hyposmotic). When the external osmolarity has dropped to 150 mOsm (50% hyposmotic), cells have lost 29% of the intracellular K⁺. A similar decrease occurs after sudden exposure to 50% hyposmotic solutions (Van Driessche et al., 1997). In contrast, in hippocampal slices whereas K⁺ is clearly mobilized during RVD, no ⁸⁶Rb efflux nor change in K⁺ occurred during IVR. This is an unexpected result because K⁺ is a key osmolyte in essentially all cell types. It should be noticed, however, that unlike in cells in culture, in the hippocampal slices having 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.

The decrease in amino acids content occurring during IVR is clearly insufficient to compensate for the change in external osmolarity and therefore, other factors have to be considered to explain the maintenance of cell volume under these conditions. One or several of the following possibilities may be considered: (1) swelling is overall restricted when the osmolarity change is small and gradual, (2) other organic osmolytes, such as creatine, myoinositol, sorbitol, or *N*-acetyl aspartate, are also contributing to counteract the external osmolarity, and altogether compensate for the initial phase of hyposmotic stress when K⁺ release has not yet still activated, (3) swelling occurs in some but not in all cells, and the decrease in amino acids and other osmolytes is required to compensate the change in cell volume only in a minor population of cells (Andrew and McVicar, 1994; Aitken et al., 1998), (4) a redistribution of osmolyte amino acids between different types of cells—i.e., neurons

and astrocytes—as has been observed in the cerebellum (Nagelhus et al., 1993), even when it may importantly contribute to regulate cell volume in specific types of cells, it may not result in a large net efflux, (5) amino acids, particularly taurine, may serve a signaling role, instead of acting directly as an osmolyte (Hussy et al., 1997), and (6) rapid metabolic changes such as synthesis of macromolecules, i.e., glycogen, may contribute to reduce the intracellular osmolyte pool necessary to reach the osmotic equilibrium (Häussinger, 1996).

Results of the present work and those in renal cells (Lohr and Grantham, 1986; Van Driessche et al., 1997) contrast with those recently reported in trout erythrocytes that do not exhibit IVR (Godart et al., 1999). In these cells, gradual changes in osmolarity failed to significantly increase the efflux of intracellular osmotically active solutes including K^+ and taurine (Godart et al., 1999). 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.

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