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Activation and Inactivation of Taurine Efflux in Hyposmotic and Isosmotic Swelling in Cortical Astrocytes: Role of Ionic Strength and Cell Volume Decrease

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A decrease in intracellular ionic strength appears involved in the activation of swelling-elicited ^{3}H-taurine efflux in cortical cultured astrocytes. Hyposmotic (50%) or isosmotic urea-induced swelling leading to a decrease of intracellular ionic strength, activated ^{3}H-taurine efflux from a rate constant of about 0.008 min^{-1} to 0.33 min^{-1} (hyposmotic) and 0.59 min^{-1} (urea). This efflux rate was markedly lower (maximal 0.03 min^{-1}) in isosmotic swelling caused by K^+ accumulation, where there is no decrease in ionic strength, or in cold (10°C) hyposmotic medium (maximal 0.18 min^{-1}), where swelling is reduced and consequently intracellular ionic strength is less affected. Also, astrocytes pretreated with hyperosmotic medium, which recover cell volume by ion accumulation, did not release ^{3}H-taurine when they swelled by switching to isosmotic medium, but when volume was recovered by accumulation of urea, taurine release was restored. These results point to a key role of ionic strength in the activation of osmosensitive ^{3}H-taurine efflux. In contrast, its inactivation was independent of the change in ionic strength but appears related to the reduction in cell volume after swelling, since despite the extent or direction of the change in ionic strength, the ^{3}H-taurine efflux did not inactivate in isosmotic KCl-elicited swelling when cell volume did not recover nor in hyposmotic swelling when RVD was impaired by replacing NaCl in the medium by permeant osmolytes. J. Neurosci. Res. 56:659–667, 1999. © 1999 Wiley-Liss, Inc.

Key words: volume regulation; osmolytes; isosmotic swelling; hyposmotic swelling; urea; KCl swelling

INTRODUCTION

Activation of pathways that translocate osmolytes during RVD requires a chain of events, including a sensor to detect the volume change and a system of transduction signals between the sensor and the effector. Subsequently, a mechanism to inactivate the osmolyte flow once the cell volume has been restored, is also required. A large number of studies have been directed to identify the nature of the osmosensitive efflux pathways (Nilius et al., 1997; Okada, 1997; Lang et al., 1998), but less is known about their mechanisms of activation and inactivation. In this work, we have addressed these questions by exploring the possible role of changes in intracellular ionic strength and of cell volume recovery in the mechanisms of activation and inactivation of swelling-induced ^{3}H-taurine efflux in rat brain cortical astrocytes in culture.

Taurine is part of the pool of organic osmolytes that contribute to cell volume regulation in a variety of cells, including brain cells (Pasantes-Morales and Schousboe, 1988; Pasantes-Morales et al., 1993). Astrocytes in culture have been widely used to examine the mechanism of regulatory volume decrease (RVD) activated after hyposmotic swelling (Kimelberg and Frangakis, 1986; Pasantes-Morales and Schousboe, 1988; Bender et al., 1992). The osmosensitive taurine release in astrocytes occurs via a leak pathway (Sánchez-Olea et al., 1991), which is blocked by a variety of anion channel inhibitors (Jackson and Strange, 1993; Sánchez-Olea et al., 1994, 1995; Hall et al., 1996), implicating an anion channel-like molecule in taurine translocation (Jackson and Strange, 1993; Roy, 1995). A decrease in intracellular ion strength has recently been considered among the signals for activation of this pathway, which might carry Cl^- as well as taurine, after swelling elicited by hyposmotic solutions (Emma et al., 1997; Nilius et al., 1998; Cannon et al., 1998). In this model of swelling, there is always a decrease in intracellular ion strength due to dilution by water entry as well as by the efflux of ionic osmolytes. This may not occur, though, when cell swelling is caused precisely by accumu-

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lation of ions. This was examined in the present work using as experimental models hyposmotic swelling and swelling in isosmotic conditions due to accumulation of KCl and urea. The effect of low temperature, which also delays and decreases the extent of swelling and the consequent change in intracellular ionic strength, was also examined. The change in intracellular ionic strength was followed considering cell K^+ levels as an approximate indication of this parameter.

Inactivation of osmolyte fluxes, including taurine, has received less atention, even though it represents also an important step in the mechanisms for cell volume control. The decrease in cell volume after osmolyte efflux was considered in this work as a possible signal for inactivation of taurine release, and the hypothesis was tested investigating the effect on the inactivation of ^{3}H-taurine efflux of conditions that prevent RVD after hyposmotic swelling or in isosmotic swelling models where RVD does not occur.

METHODS

Cell Cultures

Astrocyte cultures were prepared from neonatal (1–2 days old) rat cerebral cortex, as previously described (Pasantes-Morales et al., 1994a). The dissociated cell suspensions were plated at a density of 180 x 10^3 cells/cm^2 in 100-, 60-, or 35-mm plastic dishes or in 24-multiwell dishes. The culture medium consisted of basal Eagle's medium supplemented with 10% heat-inactivated fetal calf serum (Gibco, Gaithersburg, MD), 2 mM glutamine, 50 U/ml penicillin, and 50 µg/ml streptomycin. The culture dishes were incubated at 37°C in humidified 5% CO_2, 95% air atmosphere. The enrichment of cultures in astrocytes (95%) was assessed by immunostaining labeling with polyclonal antibodies against glial fibrillary acidic protein (astrocytes) and neuron-specific enolase (neurons) and with a monoclonal antibody against Thy-1 (fibroblasts). All cells were used after 2–3 weeks in culture.

Cell Volume Measurements

Astrocytes cultured in 60-mm dishes were washed twice with phosphate buffered saline (PBS) and detached by treatment for 3 min with PBS without calcium and containing 1 mM EDTA and 0.01% trypsin. The detached cells were mixed with the same volume of serum-containing medium, and then centrifuged and resuspended in isosmotic HEPES buffered solution containing (in mM) NaCl, 135; KCl, 5; $MgSO_4$, 0.6; $CaCl_2$, 1; glucose, 10; HEPES, 10; pH 7.4. A sample of the cell suspension was diluted approximately 100-fold with the isosmotic or hyposmotic media, and 1 min after, cell volume was measured at the indicated times by electronic sizing using a Coulter (Hialeah, FL) counter associated to a particle annalizer (Coulter channelizer). Osmolarity of all media was measured in a freezing point osmometer. Cell volume were recorded in femtoliters, but some results were expressed as relative volume, i.e., the change with time over the initial volume recorded in isosmotic medium, considering it as 1; (V/V_0).

^{3}H-Taurine Efflux

Astrocytes grown in 35-mm dishes were incubated in culture medium with ^{3}H-taurine (1 µCi/ml, 1 hr). After incubation, the culture medium was replaced by isosmotic medium and cells were superfused at a rate of 1 ml/min for 5 min, at which time ^{3}H-taurine efflux baseline was attained. Efflux was subsequently measured at indicated times in different media. The solutions were always at 37°C, except in the experiments where different temperatures were tested. Hyposmotic and hyperosmotic media were made varying the NaCl concentration. At the end of the experiment, cells were solubilized with 0.4 N NaOH and radioactivity in the collected samples and that remaining in tissue was measured by scintillation spectrometry. Results are expressed as efflux rate constants, i.e., the percentage of ^{3}H-taurine in the cells at the beginning of each sample collection period

Potassium Content Assay

Astrocytes in 100-mm dishes were treated as indicated at each experiment and then washed 4 times with ice-cold $MgCl_2$ with the same osmolarity as the experimental solutions. The K^+ content of the cells was extracted by treatment with 0.1 N HCl, and measured by flamme photometry.

RESULTS

Volume Changes, Taurine Efflux, and Intracellular K^+ Levels ($[K^+]_I$) in Hyposmotic Solutions

Figure 1A shows the effect of solutions of 50% decreased osmolarity on astrocyte volume and ^{3}H-taurine efflux. The stimulus elicited a fast cell volume increase with maximal values reached after 1 min. Immediately after this time, cells initiated RVD and cell volume decreased towards values in isosmotic conditions. The release of ^{3}H-taurine closely followed the change in cell volume, as previously described (Pasantes-Morales and Schousboe, 1988). When after 1 min of stimulation with 50% hyposmotic medium, the solution was made isosmotic by addition of NaCl, the cell volume rapidly decreased with immediate full recovery (Fig. 1B). This change in cell volume was paralleled by a rapid inactiva-

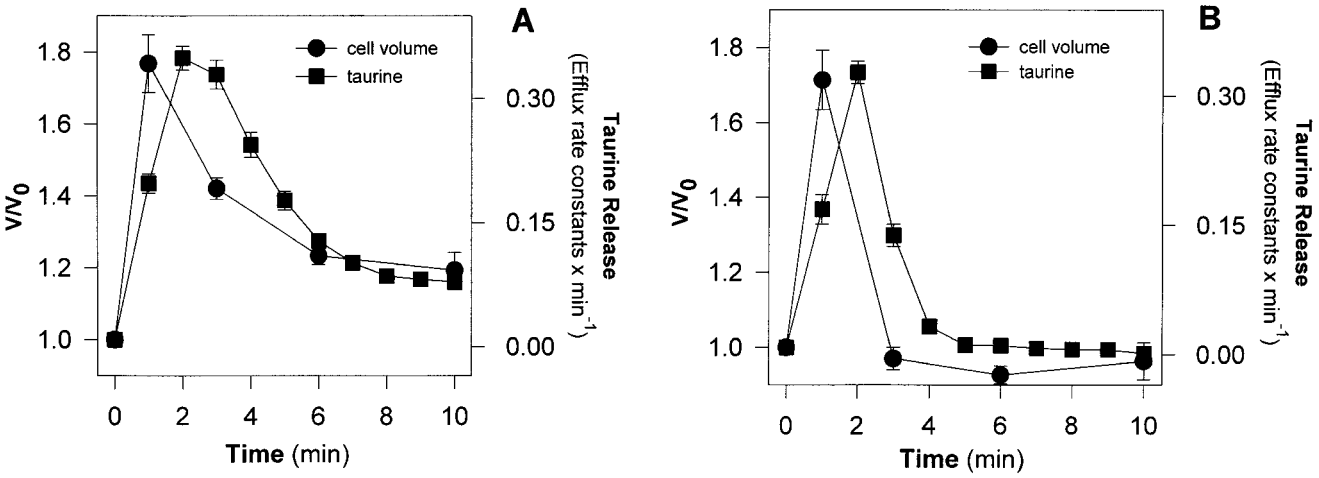

Fig. 1. Activation and inactivation of ^{3}H-taurine efflux and changes in cell volume in cultured rat cortical astrocytes. Cell cultures were maintained first in isosmotic medium (300 mOsm) and then exposed to 50% hyposmotic medium for 10 min (**A**) or during 2 min in 50% hyposmotic medium and then switched back to the isosmotic medium (**B**). The change in cell volume was assessed by electronic sizing as described in Methods and results are expressed as relative volume V/V_0. ^{3}H-taurine efflux was followed in samples collected at 1-min intervals and results are expressed as efflux rate constants calculated as described in Methods. Data are means ± SE indicated by vertical bars. (^{3}H-taurine n = 6; volume n = 4.)

tion of taurine efflux (Fig. 1B). When NaCl in the hyposmotic medium was replaced by KCl or by alanine, cells swelled normally but RVD did not occur, as previously observed, and astrocytes remained swollen (Pasantes-Morales et al., 1994a,b). Under these conditions, ^{3}H-taurine efflux did not inactivate (Fig. 2). In contrast, replacing NaCl with rafinose, a situation in which cell swelling is restricted to only 24% but RVD occurs rapidly due to an increase in K^+ efflux (Quesada et al., 1998), the inactivation of ^{3}H-taurine efflux was also accelerated (Fig. 2). In order to investigate a possible correlation between the activation and inactivation of ^{3}H-taurine efflux and the change in intracellular ion strength, the concentration of K^+ (taken as an indication of ion strength in the cell) was measured after hyposmotic swelling and during RVD in the different experimental conditions. Hyposmotic swelling is expected to reduce the $[K^+]_i$, primarily by changes in the cell water content, and also by the efflux of K^+ activated by swelling (Sánchez-Olea et al., 1993). Exposure to 50% hyposmotic medium reduced $[K^+]_i$ immediately, with a maximal change within the first 3 min. This was observed either when results are calculated as % change from the control expressed as nmol per mg protein as in Figure 3A, or in mM (mmol/l), i.e., taking into account the change in cell volume, as in Table I. After this time, as cells progressively reduced volume, $[K^+]_i$ expressed in mM, slightly increased (Table I). In cells treated with a 50% hyposmotic solution containing KCl, the K^+ cell content progressively increased with time (Fig. 3B), whereas when alanine or rafinose replaced NaCl, the loss of cell

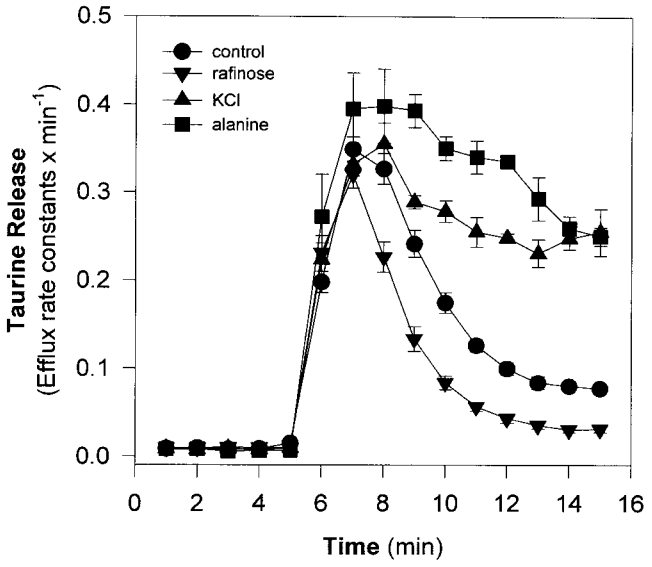

Fig. 2. Effect of changes in the rate of volume decrease (RVD) on the time course of ^{3}H-taurine efflux from cultured rat cortical astrocytes. Cells were exposed to 50% hyposmotic medium containing 60 mM NaCl (control) or equiosmolar amounts of rafinose, alanine, or KCl. Results are expressed as ^{3}H-taurine efflux rate constants calculated as in Figure 1. Data are means ± SE. (n = 4–28.)

K^+ was higher than in normal controls (Fig. 3A). As shown in Figure 2, in all cases ^{3}H-taurine efflux was rapidly activated but inactivation did not occur if cell volume was not recovered, in spite of the differences in

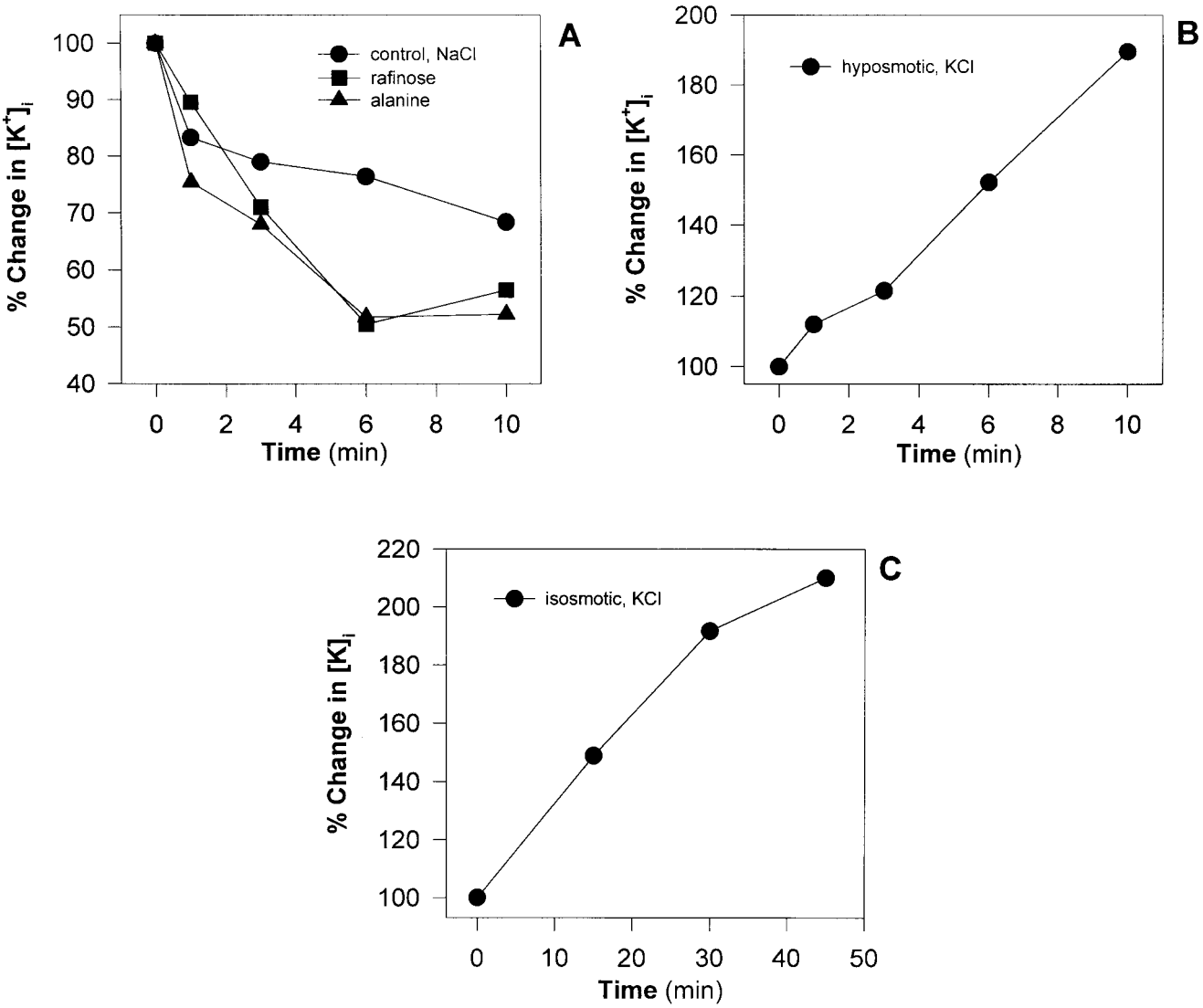

Fig. 3. Intracellular K^+ concentration ($[K^+]_i$) in rat cortical astrocytes exposed to 50% hyposmotic (**A,B**) or isosmotic (**C**) media in which NaCl was replaced by alanine, rafinose, or KCl. $[K^+]_i$ was determined by flamometry as described in Methods. A: Hyposmotic media containing 60 mM NaCl (control) or 120 mM rafinose or alanine. B: Hyposmotic medium containing 60 mM KCl. C: Isosmotic medium with all NaCl (135 mM) replaced by KCl. Results are expressed as percent change of $[K^+]_i$ in control non-stimulated cultures (101.3 mM).

cell K^+ concentration. From these results it can be concluded that in all conditions, cell K^+ levels and taurine efflux changed inversely and simultaneously, but inactivation of taurine efflux takes place only in parallel with cell volume recovery.

To further explore a possible connection between ionic strength, cell volume, and activation of ^{3}H-taurine release, the following experiment was devised. In astrocytes exposed to media made hyperosmotic (400 mOsm) by addition of NaCl, a decrease of 25.5 ± 2% in cell volume occurred within 1 min (not shown). After 4 hr in the hyperosmotic condition, cells have recovered their initial volume by accumulation of NaCl and KCl by electroneutral cotransporters (Chassande et al., 1998). Switching these cells, now having a hyperosmotic intracellular ionic content, to isosmotic medium, led to a net increase in cell volume of 27 ± 2.2% (Fig. 4B, bar 2). Despite this change in cell volume, only a marginal efflux of taurine was observed (Fig. 4A, bar 2). These results confirm the results reported in C6 glial cells (Emma et al., 1997). To further explore whether the failure of astrocytes to release taurine was due to the increase in ion strength caused by the accumulation of Na^+, K^+, and Cl^-, in cells treated as above the cotransporters were blocked by

TABLE I. Changes in $[K^+]_i$ During Isosmotic and Hyposmotic Swelling in Rat Cortical Astrocytes*

50% Hyposmotic		Isosmotic	
Time (min)	$[K^+]_i$ mM	Time (min)	$[K^+]_i$ mM
0	101.3	0	101.3
1	46.2	15	98.04
3	56.0	30	109.7
6	56.9	45	105.7
10	58.6		

*Cultured rat cortical astrocytes were exposed during the indicated times to 50% hyposmotic medium or to isosmotic KCl containing medium. After this time, cells were lysed with HCl and the K^+ content in extracts was measured by flamometry, as described in Methods. $[K^+]_i$ in mM was calculated using K^+ determinations, total amount of protein and water content per cell.

100 μM bumetanide and normal cell volume was restored by accumulation of urea. When these cells were transferred to isosmotic medium, ^{3}H-taurine release was increased (Fig. 4A, bar 3), although not to the same extent as elicited by a similar increase in cell volume in intact cells exposed to hyposmotic medium (Fig. 4A,B, bars 4).

The effect of low temperatures on hyposmotic astrocyte swelling may also be useful to examine a correlation between swelling and the consequent decrease of intracellular ionic strength, and taurine efflux. As previously shown (Pasantes-Morales et al., 1994a), whereas in 50% hyposmotic medium at 37°C maximal swelling is attained after 1 min and RVD starts immediately, at low temperatures (15°–18°C) maximal cell swelling is delayed and cell volume recovery is also affected. At 4°C, maximal swelling is not reached even after 15 min (Pasantes-Morales et al., 1994a). The efflux of ^{3}H-taurine at different temperatures observed in the present work (Fig. 5) was modified in a direction closely following that of the change in cell volume. At 37° and 42°C, the peak release was attained at about 2 min, and inactivation started immediately after, but at 20°C the peak release was delayed to min 4, inactivation started only at min 5 and proceeded slowly. At 10°C taurine efflux was very slow, showing no inactivation during the 10 min of the experiment (Fig. 5).

Volume Changes, Taurine Efflux, and $[K^+]_I$ Levels in Isosmotic Solutions

Figure 6 illustrates the change in cell volume and taurine efflux in astrocytes exposed to a solution containing 200 mM urea replacing an equiosmolar amount of NaCl. This treatment resulted in a rapid increase in cell volume due to urea accumulation followed by osmotic water. $[K^+]_i$ was correspondingly diluted. The time course of the change in cell volume elicited by isosmotic urea-containing medium was somewhat delayed in comparison with that observed in hyposmotic media but the extent of swelling was higher. The maximal volume was attained about 3 min after the stimulus and thereafter RVD progressed as in astrocytes challenged with a 50% hyposmotic solution (Fig. 6). The release of ^{3}H-taurine closely followed the time course and the magnitude of the urea-induced change in cell volume. The efflux rate constant, 0.59 min^{-1} at the peak release (Fig. 6), and the amount of ^{3}H-taurine released by cells stimulated by urea (81.4 ± 1.1%, n = 12, in 5 min) were also higher than in 50% hyposmotic medium (maximal efflux rate constant 0.33 min^{-1}, 61.6 ± 2.9%, n = 35, in 5 min). The release of taurine elicited by 200 mM urea was sensitive to the Cl^- channel blocker 5-nitro-2 (-phenylpropylamino) benzoic acid (NPPB) (taurine release 36.4 ± 3.3, n = 8, in 5 min).

A completely different pattern of volume change and taurine efflux was observed in isosmotic medium with KCl replacing equiosmolar amounts of NaCl. This condition led to a gradual volume increase with no apparent cell volume regulation within 45 min. At this time, cell volume was increased about 100% (Fig. 7). The K^+ concentration expressed per mg of protein increased with time (Fig. 7), but was unchanged when results were expressed per cell water content (Table I). The release of ^{3}H-taurine also increased, gradually and progressively, with a time course identical to the change in cell volume (Fig. 7). Taurine release expressed as efflux rate constants was notably lower, almost ten times (0.03 min^{-1}), than that elicited after an abrupt cell volume change, but since the efflux did not inactivate, after 60 min in the experimental conditions, the amount of taurine released (83.34 ± .88%, n = 26) was similar to that in 10 min in 50% hyposmotic medium (82.77 ± 1.39%, n = 30). The effects of blockers of taurine efflux elicited by hyposmotic solutions NPPB and 1,9,-dideoxyforskoline could not be tested in this model since they prevented the K^+ and Cl^- entry and the subsequent swelling.

DISCUSSION

In astrocytes, as in many other cell types, hyposmotic swelling leads to activation of taurine efflux, with a magnitude proportional to the extent of the change in cell volume (Pasantes-Morales and Schousboe, 1988; Pasantes-Morales et al., 1993). This experimental model of swelling always results in a decrease in the intracellular ionic strength, due in part to the dilution of the ionic content of the cell by water entry and also by the activated exit of K^+ and Cl^-. Motais et al. (1991) first pointed out a prominent role of cell ionic strength in the mechanisms responsible for the osmosensitive release of taurine in trout red cells. More recently, ionic strength has been suggested as a modulator of the taurine efflux pathway

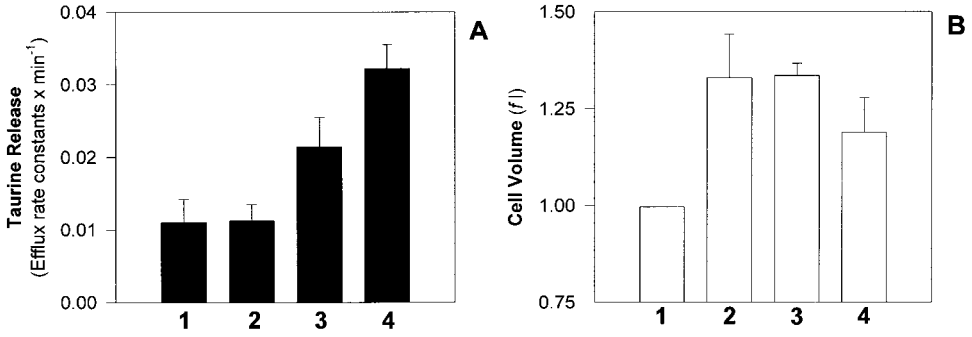

Fig. 4. Effects of modifications of intracellular ion strength on ^{3}H-taurine release (**A**) and cell volume (**B**) in rat cortical astrocytes. For each experiment of this set, four groups of cells were exposed during 4 hr to the following media: 1,4: isosmotic medium; 2: hyperosmotic medium (400 mOsm, NaCl 185mM), 3: hyperosmotic medium (400 mOsm) plus 100 μM bumetanide. At the end of this period, media in 1 and 2 were replaced by isosmotic solution and ^{3}H-taurine efflux and the change in cell volume were measured after one minute. In 3, astrocytes were incubated 5 min in hyperosmotic medium containing 135 mM NaCl and 100 mM urea (400 mOsm) plus 100 μM bumetanide and then in isosmotic solution, before ^{3}H-taurine release and cell volume were measured. In 4, astrocytes were exposed to 20% hyposmotic medium. (^{3}H-taurine release n = 3–14; cell volume n = 3.)

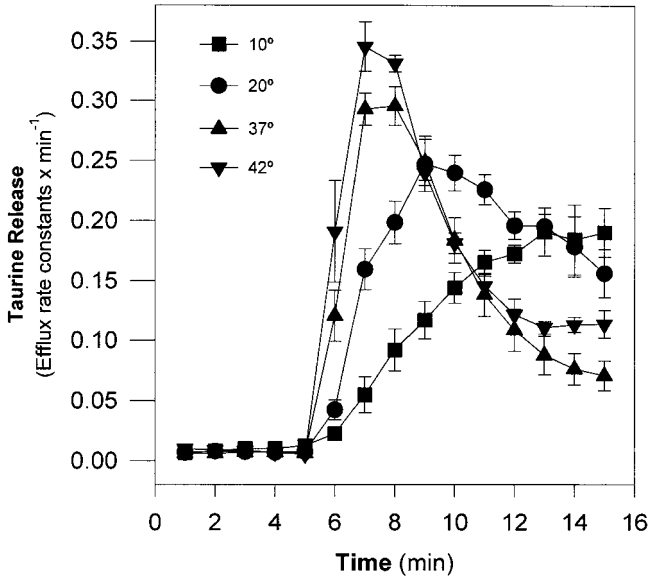

Fig. 5. Effect of temperature on ^{3}H-taurine release in rat cortical astrocytes. Cells were preincubated 5 min in isosmotic medium at the indicated temperatures, then exposed to 50% hyposmotic media at the same temperature. ^{3}H-taurine efflux measured and expressed as in Figure 1. Data are means ± SE (n = 4–7).

sensitivity in C6 glioma cells (Emma et al., 1997). In this study, glioma cells shrunken by treatment with hyperosmotic solutions, accumulate K^+, Na^+, and Cl^- to increase volume up to normal values. When these cells are switched to an isosmotic medium, they swell as a consequence of the hyperosmotic intracellular composition, but despite the dilution, the cell ionic strength remains above normal levels. Under these conditions, taurine efflux is not activated. This is only restored when after some time, within 12 and 24 hr, organic osmolytes replace intracellular ions and, then, a decrease in ionic strength below normal levels occurs upon swelling in isosmotic medium. The delay for changing the intracellular content of hyperosmotically adapted cells from ions to organic osmolytes is that required for full adaptation of the organic osmolyte transporters to the new situation. However, the hyperosmotic treatment may affect other mechanisms also involved in taurine efflux, such as interactions with the cytoskeleton, which could be readapted during this long period. This is why in our study we made this change in volume occur immediately by, first, avoiding ion accumulation with bumetanide and, second, by rapidly restoring the cell volume with a readily permeable organic compound such as urea. We found that astrocytes, treated as C6 cells, react in the same way, i.e., when exposed to hyperosmotic solutions that accumulate external ions for the active volume recovery, swelling by switching to isosmotic medium only results in a small increase of taurine efflux, despite a change in cell volume that regularly activates its release to a large extent. After the above-described experimental maneuvers, astrocytes recovered the ability to release taurine. Another result emphasizing the importance of a decrease in ionic strength for taurine efflux was the response of cells stimulated by rafinose-containing hyposmotic medium, where a decrease of only 10% in K^+ content was sufficient to fully activate the release of taurine, even when cell swelling was notably low. Moreover, the markedly low rate of taurine efflux observed in isosmotic swelling due to accumulation of KCl (Walz, 1987), with no decrease in ionic strength, further stresses the correlation between the two phenomena. However,

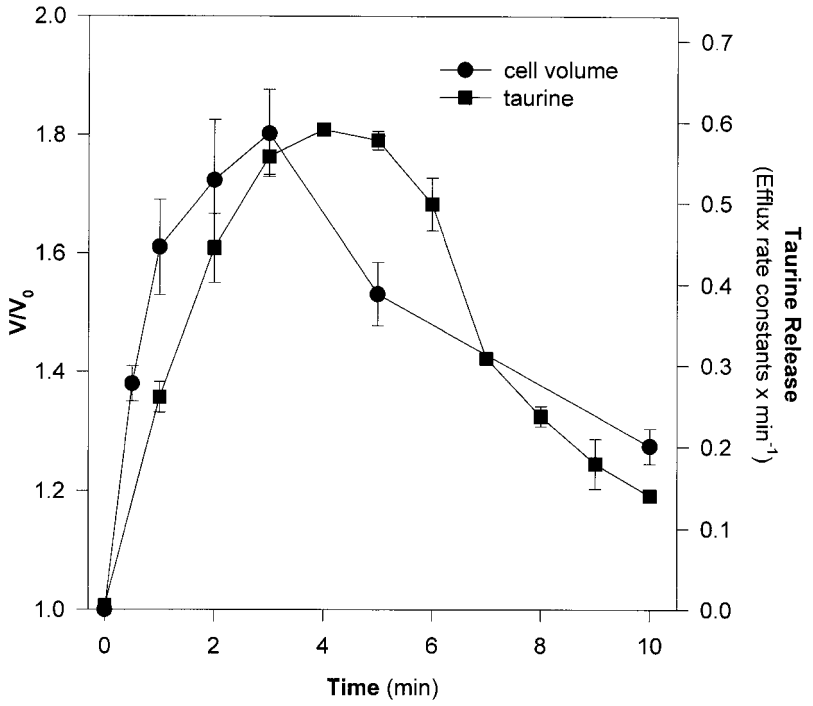

Fig. 6. Effect of urea containing isosmotic medium on cell volume and ^{3}H-taurine release in rat cortical astrocytes. After loading in normal isosmotic medium, cells were incubated in the experimental medium and samples for measurement of ^{3}H-taurine release and cell volume were taken at the indicated times. Urea containing medium was prepared replacing 100 mM of NaCl for 200 mM urea. Data are means ± SE (n = 6).

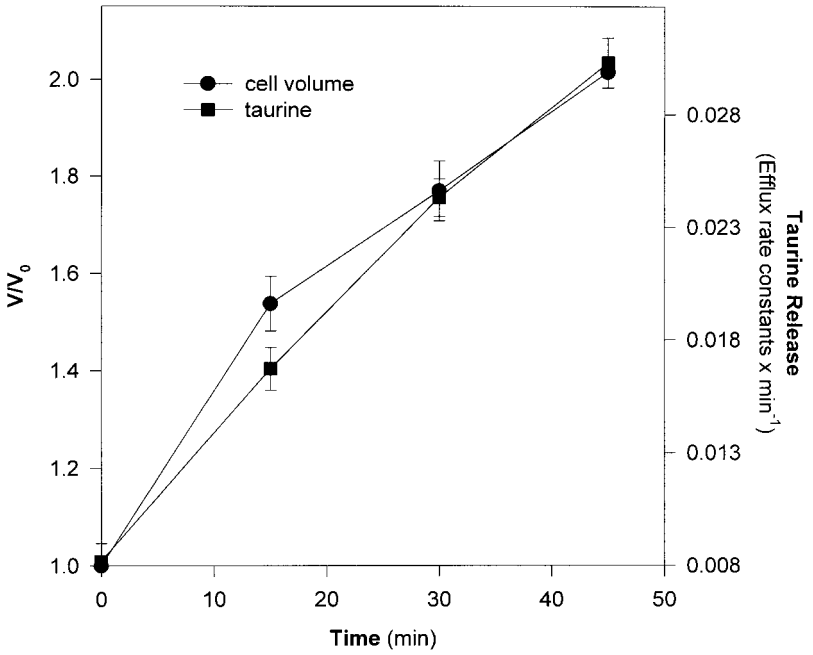

Fig. 7. Effect of KCl containing isosmotic medium on cell volume and ^{3}H-taurine efflux in rat cortical astrocytes. After loading in normal isosmotic medium, cells were incubated in the experimental medium and samples for measurement of ^{3}H-taurine release and cell volume were taken at the indicated times. KCl containing medium was prepared replacing 135 mM NaCl for 135 mM KCl. Data are means ± SE (^{3}H-taurine n = 28; volume n = 4).

other differences such as the rapid, abrupt change in cell volume in hyposmotic solutions and the gradual, slow increase in isosmotic KCl-containing medium, may somehow influence the taurine release pattern. Organization of the cytoskeleton, for instance, which may be implicated in the activation of the taurine efflux pathway, could respond differently in such distinct patterns of change in cell volume. It is noteworthy that the activation of the volume-sensitive Cl^- channel, which appears to be identical to the taurine leak pathway, also depends on these changes of ionic strength in C6, CHO, and endothelial cells (Emma et al., 1997; Cannon et al., 1998; Nilius et al., 1998).

Inactivation of taurine efflux appears independent of the change in ion strength but seems markedly dependent on the cell volume. This was shown by

experiments in which preventing RVD and the decrease in cell volume (Pasantes-Morales et al., 1994a,b), by replacing NaCl with KCl or alanine in the hyposmotic media, taurine efflux did not inactivate, regardless of the change in ionic strength, which decreased with alanine and increased with high KCl. Also, no inactivation of taurine efflux was observed in isosmotic solutions containing high K^+ concentrations, with astrocytes continuously swelling, leading to depletion of the cell taurine pool. From these results, it appears that cell volume and not ionic strength is a determinant for inactivation of the swelling-sensitive taurine efflux.

It has been demonstrated that the osmosensitive taurine pathway is a leak pathway, not involving energy-dependent carriers (Sánchez-Olea et al., 1991; Kirk, 1997). Despite this, the efflux of taurine from cortical astrocytes was found markedly dependent on temperature, being reduced and delayed at 10°–20°C. A similar sensitivity to temperature has been described for the volume-activated taurine release in MDCK cells (Sánchez-Olea et al., 1991) and for Cl^- efflux in Ehrlich ascites cells (Riquelme et al., 1998). In a previous study in astrocytes (Pasantes-Morales et al., 1994a) we found that cell swelling and RVD were also profoundly influenced by temperature. At low temperatures (15°–18°C) maximal cell swelling is delayed and cell volume recovery is also affected. At 4°C, maximal swelling is not reached even after 15 min, with no occurrence of RVD at this time (Pasantes-Morales et al., 1994a). In the present study, activation of taurine efflux at low temperatures was also delayed, possibly because the decrease in ionic strength by dilution is also restricted. Inactivation of taurine efflux was also changed according to the delay of cells to recover volume. Besides these possible correlations, an effect of temperature on metabolic events such as phosphorylation reactions, particularly on tyrosine kinases, which appear implicated in the mechanism of the volume-sensitive Cl^- efflux (Crépel et al., 1998; Tilly et al., 1993; Va der Wijk et al., 1998), should not be excluded for taurine, considering the similarities between this channel and the taurine pathway. Interestingly, there is also information about the influence of ionic strength on phosphorylation reactions such as those involved in the interactions between synapsin and synaptic vesicles (Stefani et al., 1997) or on the binding to the cytoskeleton of non-receptor tyrosine kinases present in the nerve growth cones (Helmke and Pfenninger, 1995). A better understanding of the complex interactions between the chain of transducing signals, the cytoskeleton elements, and the efflux pathways, will provide more detailed information about the site of influence of the factors examined in this work.

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REFERENCES

Bender AS, Neary JT, Norenberg MD. 1992. Involvement of second messengers and protein phosphorylation in astrocyte swelling. Can J Physiol Pharmacol 70:S362–S366.

Cannon CL, Basavappa S, Strange K. 1998. Intracellular ionic strength regulates the volume sensitivity of a swelling-activated anion channel. Am J Physiol 275:C416–C422.

Chassande O, Frelin C, Farahifar D, Jean T, Lazdunski M. 1998. The $Na^+/K^+/Cl^-$cotransport in C6 glioma cells. Properties and role in volume regulation. Eur J Biochem 171:425–433.

Crépel V, Panenka W, Kelly MEM, MacVicar B. 1998. Mitogen-activated protein and tyrosine kinases in the activation of astrocyte volume-activated chloride current. J Neurosci 18:1196–1206.

Emma F, McManus M, Strange K. 1997. Intracellular electrolytes regulate the volume set point of the organic osmolyte/anion channel VSOAC. Am J Physiol 272:C1766–1775.

Hall JA, Kirk J, Potts JR, Rae C, Kirk K. 1996. Anion channel blockers inhibit swelling-activated anion, cation, and nonelectrolyte transport in HeLa cells. Am J Physiol 271:C579–C588.

Helmke S, Pfenninger KH. 1995. Growth cone enrichment and cytoskeletal association of non-receptor tyrosine kinases. Cell Motil Cytoskeleton 30:194–207.

Jackson PS, Strange K. 1993. Volume-sensitive anion channels mediate swelling-activated inositol and taurine efflux. Am J Physiol 265:C1489–C1500.

Kimelberg HK, Frangakis MV. 1986. Volume regulation after swelling in primary astrocyte cultures. Adv Biosci 61:177–186.

Kirk K 1997. Swelling-activated organic osmolyte channels. J Membr Biol 158:1–16.

Lang F, Busch GL, Ritter M, Volkl H, Waldegger S, Gulbins E, Haussinger D. 1998. Functional significance of cell volume regulatory mechanisms. Physiol Rev 78:247–306.

Motais R, Guizouarn H, Garcia-Romeu F. 1991. Red cell volume regulation: the pivotal role of ionic strength in controlling swelling-dependent transport systems. Biochim Biophys Acta 1075:169–180.

Nilius B, Eggermont J, Voets T, Buyse G, Manolopoulos V, Droogmans G. 1997. Properties of volume-regulated anion channels in mammalian cells. Prog Biophys Mol Biol 68:69–119.

Nilius B, Prenen J, Voets T, Eggermot J, Droogmans G. 1998. Activation of volume-regulated chloride currents by reduction of intracellular ionic strength in bovine endothelial cells. J Physiol (Lond) 506:353–361.

Okada Y. 1997. Volume expansion-sensing outward-rectifier Cl^- channel: fresh start to the molecular identity and volume sensor. Am J Physiol 273:C755–C789.

Pasantes-Morales H, Schousboe A. 1988. Volume regulation in astrocytes: a role for taurine as osmoeffector. J Neurosci Res 20:505–509.

Pasantes-Morales H, Alavéz S, Sánchez-Olea R, Morán J. 1993. Contribution of organic osmolytes to volume regulation in rat brain cells in culture. Neurochem Res 18:445–452.

Pasantes-Morales H, Murray RA, Lilja L, Morán J. 1994a. Regulatory volume decrease in cultured astrocytes I. Potassium- and chloride-activated permeability. Am J Physiol 266:C165–C171.

Pasantes-Morales H, Murray RA, Sánchez-Olea R, Morán J. 1994b. Regulatory volume decrease in cultured astrocytes II. Activated permeability to amino acids and polyalcohols. Am J Physiol 266:C172–C178.

Quesada O, González E, Morales-Mulia S, Bargas J, Pasantes-Morales H. 1998. Effects of NaCl removal on osmolyte fluxes and regulatory volume decrease in cultured astrocytes. J Neurosci Res 53:195–202.

Riquelme G, Sepúlveda FV, Jorgensen F, Pedersen S, Hoffman EK. 1998. Swelling-activated potassium currents of Ehrlich ascites tumour cells. Biochim Biophys Acta 1371:101–106.

Roy G. 1995. Amino acid current through anion channels in cultured human glial cells. J Membr Biol 147:35–44.

Sánchez-Olea R, Morán J, Schousboe A, Pasantes-Morales H. 1991. Hyposmolarity- activated fluxes of taurine in astrocytes are mediated by diffusion. Neurosci Lett 130:233–236.

Sánchez-Olea R, Morán J, Martínez A, Pasantes-Morales H. 1993. Volume-activated Rb^+ transport in astrocytes in culture. Am J Physiol 264:C836–C842.

Sánchez-Olea R, Peña C, Morán J, Pasantes-Morales H. 1994. Inhibition of volume regulation and efflux of osmoregulatory amino acids by blockers of Cl transport in cultured astrocytes. Neurosci Lett 156:141–144.

Sánchez-Olea R, Morales-Mulia M, Morán J, Pasantes-Morales H. 1995. Inhibition by polyunsaturated fatty acids of regulatory volume decrease and osmolyte fluxes in astrocytes in culture. Am J Physiol 269:C96–C102.

Stefani G, Onofri F, Valtorta F, Vaccaro P, Greengard P, Benfenati F. 1997. Kinetic analysis of the phosphorylation-dependent interactions of synapsin I with rat brain synaptic vesicles. J Physiol (Lond) 504:501–515.

Tilly BC, van den Berghe N, Tertoolen LGJ, Edixhoven MJ, de Jonge HR. 1993. Protein tyrosine phosphorylation is involved in osmoregulation of ionic conductances. J Biol Chem 7:1419–1427.

Walz W. 1987. Swelling and potassium uptake in cultured astrocytes. Can J Physiol Pharmacol 65:1051–1057.

Van der Wijk T, Dorrestijn J, Narumiya S, Maassen JA, de Jonge HR, Tilly BC. 1998. Osmotic swelling-induced activation of the extracellular-signal-regulated protein kinases Erk-1 and Erk-2 in intestine 407 cells involves the Ras/Raf-signalling pathway. Biochem J 331:863–869.