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Amino Acid Osmolytes in Regulatory Volume Decrease and Isovolumetric Regulation in Brain Cells: Contribution and Mechanisms

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Key Words
Hyponatremia • Taurine • Osmolytes • Brain edema • Swelling

Abstract
Brain adaptation to hyposmolarity is accomplished by loss of both electrolytes and organic osmolytes, including amino acids, polyalcohols and methylamines. In brain in vivo, the organic osmolytes account for about 35% of the total solute loss. This review focus on the role of amino acids in cell volume regulation, in conditions of sudden hyposmosis, when cells respond by active regulatory volume decrease (RVD) or after gradual exposure to hyposmotic solutions, a condition where cell volume remains unchanged, named isovolumetric regulation (IVR). The amino acid efflux pathway during RVD is passive and is similar in many respects to the volume-activated anion pathway. The molecular identity of this pathway is still unknown, but the anion exchanger and the phospholemman are good candidates in certain cells. The activation trigger of the osmosensitive amino acid pathway is unclear, but intracellular ionic strength seems to be critically involved. Tyrosine protein kinases markedly influence amino acid efflux during RVD and may play an important role in the transduction signaling cascades for osmosensitive amino acid fluxes. During IVR, amino acids, particularly taurine are promptly released with an efflux threshold markedly lower than that of K^+, emphasizing their contribution (possibly as well as of other organic osmolytes) *vs* inorganic ions, in the osmolarity range corresponding to physiopathological conditions. Amino acid efflux also occurs in response to isosmotic swelling as that associated with ischemia or trauma. Characterization of the pathway involved in this type of swelling is hampered by the fact that most osmolyte amino acids are also neuroactive amino acids and may be released in response to stimuli concurrent with swelling, such as depolarization or intracellular Ca^{++} elevation.

Introduction

Cell volume perturbation is a challenge for homeostasis in all animal organs, but has particularly dramatic consequences in brain. The limits to expansion imposed by the rigid skull give narrow margins for the

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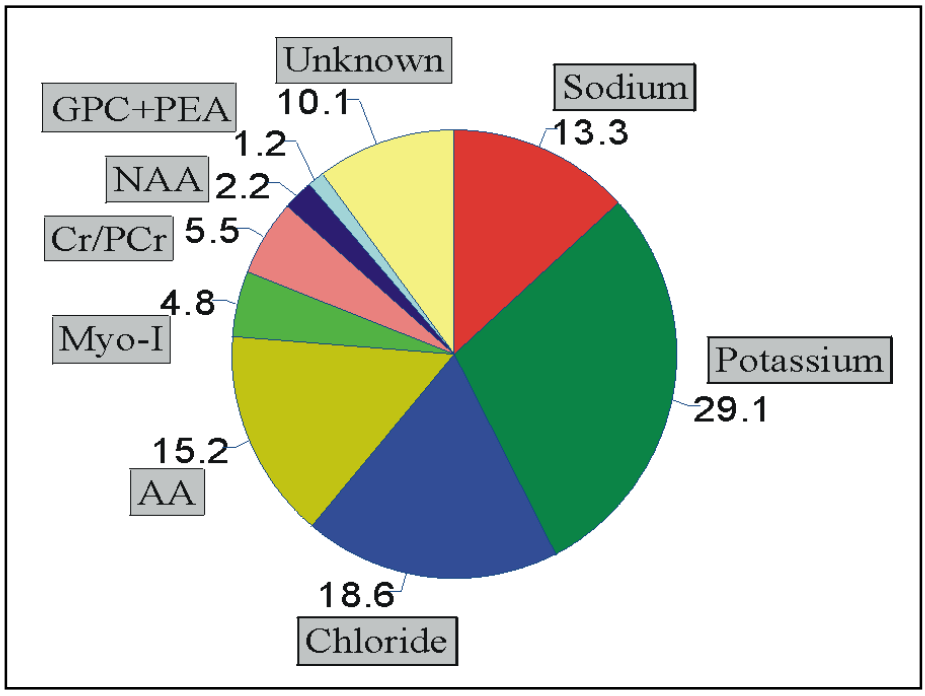

Fig. 1. Contribution of different types of osmolytes to volume adjustment in brain in vivo during chronic hyponatremia. Recalculated from: Lien et al, 1991; Verbalis and Gullans, 1991, 1993; Lien, 1995; Sterns et al, 1993. Glycero-phosphoryl choline (GPC), Phosphoetanolamine (PEA), N-acetylaspartate (NAA), Creatine (Cr), Phosphocreatine (PCr), Myo-inositol (Myo-I) and Amino acids (AA).

buffering of intracranial volume changes, leading to compression of small vessels generating episodes of anoxia and ischemia. As pressure increases, herniation leads to respiratory and cardiac arrest. Besides these extreme effects, brain cell swelling may also lead to hyperexcitability [1]. Early studies in chronic hyponatremia, showed that brain does not behave as a perfect osmometer, and the initial swelling is followed by progressive water loss until almost complete normalization, despite the persistence of hyponatremia. The observed electrolyte decrease was not sufficient to compensate for the loss of water and evidence was then obtained pointing to a significant contribution of organic osmolytes, including the most abundant amino acids, as well as of N-acetylaspartate, myo-inositol, creatine, phosphocreatine, phosphoetanolamine and glycero-phosphoryl choline [2, 3] (Fig. 1). In rodents, taurine is the most important organic osmolyte, because it is highly concentrated and shows the largest reduction during hyponatremia. In other species with lower brain taurine content, compounds such as N-acetylaspartate, may have an important role [4]. The estimation of organic osmolyte change in all these studies does not discriminate neither the regional variation within the brain nor possible differences in the cell type. *In vitro* studies in tissue slices as well as in cells in culture exposed to hyposmotic media represent a convenient model to address these questions.

Amino acids and regulatory volume decrease (RVD) during hyposmosis

Activation and inactivation of corrective fluxes

In cultured astrocytes and neurons, hyposmosis leads to rapid swelling followed by typical RVD. The efflux pattern of amino acids closely parallels the time course of the change in cell volume [5] in contrast to Cl^- and K^+ fluxes which are faster (Cl^-) or slower (K^+) than the change in cell volume. Osmosensitive efflux of amino acids has also been reported in hippocampal and cortical slices [6, 7] and in vivo during continuous superfusion of cerebral cortex [8] or by microdialysis [9]. In all these preparations, taurine is the most sensitive to the osmotic perturbation, with the lowest release threshold and the largest amount released. Interestingly, in the neuroblastoma cell line CHP-100, glutamate is not responsive to hyposmosis [10].

The amino acid efflux pathway

A leak pathway rather than an energy-dependent cotransporter, was first suggested as the mechanism for osmosensitive taurine release in Ehrlich ascites cells [11] and this was confirmed in brain cells, in which taurine translocation is passive, directed only by the concentration gradient. [12]. Unexpectedly, taurine efflux was found sensitive to Cl^- channel blockers [5], and this led to propose an anion channel-like molecule as the transport pathway for Cl^- and organic osmolytes [13]. The Cl^- channel involved in RVD is a volume-sensitive outwardly rectifying Cl^- channel (VSCC) of broad spectrum, permeable to most monovalent anions, and to some extent to large anions and to organic anions [14-17]. The properties of VSSC have been recently reviewed in detail [14, 15]. In brain cells, this channel has been characterized in the C6 glioma cell line [13], in cerebellar granule neurons [17] and in the N2A neuroblastoma [18]. Evidence in support of this VSCC as the common pathway for organic osmolytes is rather indirect, based essentially on the similar pharmacological profile of swelling-activated Cl^- currents and the swelling-induced osmolyte release [5, 13]. Currents carried through VSCC by glutamate, taurine and aspartate in the anion form, have been observed in MDCK, glioma and IMCD cells [19, 20]. Although these experiments do not prove the transport of neutral amino acids, they at least demonstrate that the size of the pore is sufficiently large for the passage of amino acids. Against this common pathway are findings of cell lines exhibiting Cl^- channel but not taurine fluxes and vice versa [16]. Also, different actions of blockers (arachidonic acid and DIDS) suggest different pathways [21, 22]. Swelling-induced taurine release without chloride channel activity in oocytes expressing anion channels and transporters also strongly favors the idea of separate pathways for taurine and Cl^- [23]. If this is the case, it should be a remarkable similarity between the molecular species permeating the two types of osmolytes, or a close interconnection between the fluxes of Cl^- and organic osmolytes.

Osmosensitive taurine fluxes appear to be carried by the anion exchanger in fish but not in mice erythrocytes [24, 25]. The protein domains responsible for the differences between mouse and trout anion exchangers have been identified, thus opening the possibility to select the anion exchanger forms in different cell types by the presence of these protein domains [26]. The phospholemman another canditate for taurine transport is a member of a superfamily of proteins with single transmembrane domains exhibiting markedly high permeability to taurine, a feature possibly due to the presence of binding sites for cations and anions within the pore. Phospholemman is present in cultured astrocytes and neurons. Overexpression in HEK cells increases RVD, osmosensitive Cl^- currents and taurine fluxes [27, 28].

Activation and transduction signalling cascades

The trigger to activate the osmosensitive Cl^-/amino acid pathways and the identification of transduction signalling cascades are still unresolved. Available information refers mainly to the volume-activated Cl^- currents, with scarce confirmation about similarities or dissimilarities with the amino acid efflux pathways. Hyposmotic swelling leads to changes in the concentration of second messengers, such as Ca^{2+}, cAMP, IP3 and arachidonic acid [29]. It is still undefined whether these signalling systems are part of the mechanisms directly activating the osmolyte corrective fluxes or occur in connection with the set of other cell responses generated by hyposmosis. Cell swelling and RVD are complex phenomena involving cell reactions to stress, reorganization of the cytoskeleton, and adhesion or retraction mechanisms, among others. All of them activate their own signals, which may or may not be implicated in the activation of corrective osmolyte fluxes.

Calcium and calmodulin

Hyposmotic swelling is associated with an increase in $[Ca^{2+}]i$ levels, which occurs also in brain cells. However, with few exceptions, Cl^- currents and osmosensitive taurine fluxes are essentially Ca^{2+}-independent [30], although a minimal amount of cell Ca^{2+} (>50 nM), referred as permissive Ca^{2+}, appears necessary for the activation of Cl^-/taurine osmosensitive fluxes [31]. It might thus be that the swelling-induced $[Ca^{2+}]i$ increase is an epiphenomenon, unrelated to the corrective fluxes of osmolytes. Taurine fluxes are blocked by pimozide and trifluoperazine [6] but their effects appear unrelated to calmodulin systems since the inhibition persists in cells where taurine efflux is Ca^{2+}-independent or even in those where swelling does not elicit any Ca^{2+} increase.

Protein kinases (PK)

PKC, PKA and cAMP. The osmosensitive amino acid fluxes appear largely PKC independent, as shown by the failure of PKC blockers or to maneuvers directed

to activate or down regulate the enzyme [32]. Taurine fluxes are PKC-independent in rat supraoptic nucleus and in cultured cerebellar granule neurons [33, 34]. Swelling- and stress-induced changes in cAMP levels have been reported in some but not in all cell types [15]. cAMP potentiates osmosensitive taurine efflux in C6 cells and in brain cortex [32, 35], but not in cerebellar granule neurons nor in supraoptic nucleus slices [33, 34]. Information about modulation of VSCC by protein kinases is reviewed in detail in [14, 15].

Protein tyrosine kinases

A role for protein tyrosine kinases (PTK) in the volume-related signalling is suggested by the numerous PTK which phosphorylate by swelling: $p125^{FAK,}$, p38, JNK, $p56^{lck}$, $p72^{syk}$ and ERK1/ERK2 [36-40]. Further support comes from the potent inhibitory effect of PTK blockers on Cl^- and taurine fluxes and the corresponding potentiation by the tyrosine phosphatase blocker o-vanadate [34, 36]. The specific kinases involved in amino acid release and the precise step of reaction have not been identified. Swelling activation of PTK does not necessarily imply a link with osmolyte fluxes, as occurs for ERK1/ERK2, for which prevention of the hyposmosis-induced phosphorylation has no effect on taurine fluxes or Cl^- currents [34, 36]. The same lack of correlation is found for the stress-activated protein kinase p38 [41]. This dissociation suggests the involvement of some PTK in phenomena coincident with swelling, but not necessarily in the activation of osmosensitive fluxes. However, such correlation may be cell specific, as shown for ERKs phosphorylation and the osmosensitive Cl^- current in cortical astrocytes [37]. Swelling-induced activation of p56lck in lymphocytes is required for VSCC functioning as p56lck deficiency by genetic knockout, leads to defective VSCC and RVD, a condition reversed by retransfection of the protein [39]. Swelling-induced tyrosine phosphorylation of band 3 (anion exchanger) in skate erythrocytes is also linked to $p72^{syk}$ and $p56^{lyn}$ [42]. This is the first report showing direct tyrosine phosphorylation of the osmolyte translocation pathway.

Tyrosine kinases and cytoskeleton

Cell swelling and RVD require a substantial reorganization of the cytoskeleton, to cope with the changes in cell volume and cell adhesion, but it is unclear whether these changes are directly involved in activation of osmolyte fluxes. A connection may be established through p21Rho, which is closely involved in the reorganization of the actin cytoskeleton and also modulates osmosensitive Cl^- currents [43, 44]. Downstream Rho, two possibilities have been explored, one of them suggesting a link with $p125^{FAK}$ and PI3 kinase, and another one proposing Rho kinase as the downstream target [43, 44]. Manipulation of these pathways has clear effects on VSCC, but less is known about their influence in amino acid fluxes. A cytoskeleton connection with taurine is suggested by decreased hyposmotic taurine efflux in astrocytes from vimentin/GFAP-deficient mice as compared to cells from the wild type mice [45].

PI3 kinase

Hyposmosis activates PI3K in some cells, and blockade by wortmannin, LY294002 or antibodies to the 110-catalytic subunit impairs cell volume recovery, VSCC activation, and the osmosensitive I^{125} and taurine fluxes [34, 43, 46]. In cerebellar granule neurons, wortmannin but not LY294002, decreases the osmosensitive taurine efflux [34]. This difference may be due to permeability restrictions to LY294002 or to different sensitivity of PI3K isoforms. However, results based only on effects of wortmannin should be taken with caution, further considering that wortmannin may also affect phospholipase A and the myosin light chain, two proteins which also appear involved in osmolyte fluxes [47].

Phosholipases (PL)

Implication of PLAs in osmolyte fluxes came from the early work by Hoffman, Lambert and coworkers [48] in Ehrlich ascites cells, showing an effect of leukotrienes LTC4 and LTD4 accelerating RVD and enhancing taurine efflux under isotonic conditions. This may not be a general mechanism of osmolyte activation since LTD4 does not affect the osmosensitive Cl^- currents in many other cell types [14,15]. However, PLA2 may still modulate the taurine/Cl efflux pathways as shown by a study in neuroblastoma CP100 cells, where swelling increases arachidonic acid release, which, if prevented by AACOCF3, inhibits taurine and Cl^- fluxes [49]. At variance with these results in isolated cells, in rat brain cortex in vivo, amino acid fluxes are essentially unaffected by the PLA2 blockers pBPB, DEDA and AACOCF3 [32].

The connection between all these enzymes remains to be established. PI3K is a key intermediate in signaling cascades, interacting notably with the Rho GTP-binding proteins, which as discussed above, appear to criti-

cally influence VSCC. An association between PI3K, Rho GTPases and phospholipases has been shown in a variety of pathways, some of them regulating the dynamics of the cortical and cytoplasmic actin cytoskeleton. A possibility that cannot be ruled out is that PLA2 is acting at the very early steps of the signalling cascades acting as a volume sensor, since there is some evidence in support of PLA2 as a mechanosensor [50].

The influence of ionic strength

The importance of intracellular ionic strength as a regulatory signal for activation of taurine fluxes in trout erythrocytes was first proposed by Motais et al. [51]. Consistent with this proposal are findings in C6 glioma and in CHO cells showing that, as intracellular ionic strength increases, larger volume changes are progressively required to activate taurine efflux [52]. In cortical astrocytes, taurine efflux is notably higher when swelling decreases ionic strength as in hyposmotic- or urea-induced swelling, as compared with K-generated swelling occurring without a decrease in ionic strength [53]. In CPAE cells, a Cl^- current identical to that elicited by hyposmotic swelling is activated by reducing the ionic strength at constant osmolarity**.** All these results suggest an effect of intracellular ionic strength either shifting the volume set point [52] or directly acting as activation signal [54]. A recent study in skate red blood cells confirms the above results, but in addition, demonstrate the influence of ionic strength on the activity of some PTK directly involved in the activation of taurine fluxes [55].

Amino acids and isosmotic swelling

Brain cell edema in isosmotic conditions (also called cytotoxic edema) conveys more risks than hyposmotic swelling, since in cytotoxic swelling there is no clear evidence of efficient cell volume correction. Ischemic stroke, head trauma and hepatic encephalopathy, are pathological conditions associated with brain edema, leading to a critical clinical challenge. Swelling also occurs in excitotoxicity and seizures [56]. The mechanisms generating swelling may be somewhat different in each pathology, but in all cases, the influx of anions, Cl^- or/ and bicarbonate-, is a consistent causal factor.

As mentioned above, in brain cells adaptive mechanisms during isosmotic swelling appear less efficient than in hyposmotic swelling. This may have to do with a difficulty for ionic osmolytes to be released when ion accumulation is the condition generating swelling. In this case, the contribution of organic osmolytes may not be sufficient to regulate cell volume, although it is certainly important to attenuate the magnitude of swelling. Amino acid efflux during cytotoxic swelling has been observed in experimental models of ischemia, from *in vitro* chemical models or *in vivo*, by vein occlusion. Cell exposure to high K^+ concentrations, as occurs in most situations leading to cytotoxic swelling, is often used to generate cytotoxic edema. Activation of the various excitatory receptor subtypes by glutamate, kainate and other agonists, are also experimental models simulating cytotoxic swelling. In all these cases, fluxes of taurine, glutamate, GABA and glycine, consistently increase. However, it should be mentioned that these amino acids are all neuroactive compounds, and some of them are important synaptic transmitters. Therefore, when evaluating the effect of cytotoxic edema on amino acid release, it is necessary to discriminate between a pure response to swelling and that related to other signals, as depolarization or Ca^{2+} entry, concurrent with ischemia, epileptic activity or excitotoxicity. Conversely, increase of extracellular K^+ or glutamate concentration known to occur in ischemia or epileptic episodes, and currently attributed to neuronal hyperexcitability, may rather be a response to swelling. This point has been addressed recently in studies about the mechanism of glutamate efflux in ischemia. Two possibilities have been considered. One explains the increase in extracellular glutamate concentration as a result of swelling-activated corrective efflux [56, 57]. The other one implicates a reverse operation of the energy-dependent glutamate transporters, due to intracellular Na^+ accumulation resulting from the energy failure, and thus, unrelated to swelling. Both possibilities have experimental support, but the option involving an impaired transport mechanism is more favored at present [58]. An effect of swelling on glutamate transporters has been described, which may link the two options [56]. The same situation applies for other amino acids such as aspartate, GABA, taurine and glutamine, which are also released in ischemic conditions, and are also transported by energy-dependent carriers [59-61]. Depolarization concurrent with ischemia may also trigger amino acid release. Differences may exist, though, in the relative sensitivity of amino acids to swelling or depolarization, which may be useful to estimate their role

as osmolytes in cytotoxic edema. In any event, the release of inhibitory amino acids such as taurine, GABA or glycine, which in contrast to glutamate, do not generate *per se* a secondary volume increase, nor excitotoxicity, may contribute to attenuate swelling and additionally, to counteract the hyperexcitability generated by K^+ and glutamate.

Studies in ischemic models of vein occlusion, have shown a blockade of amino acid fluxes by anion channel inhibitors, as in hyposmotic swelling, suggesting similar translocation pathways in the two conditions [61,62]. However, it is worthy to emphasize that these agents affect most Cl^- channel types and may affect Cl^- influx which is an essential causal element of swelling. Therefore, a reduced efflux of amino acids may be due, not to an effect on the efflux pathway, but a consequence of less swelling by Cl^- influx reduction. Cytotoxic swelling associated with hyperamonemia or with head trauma also involves an increased efflux of amino acids, including excitotoxic amino acids. The mechanism of this release is still unclear, but at least for glutamate and taurine, it seems not directly related to a swelling-induced efflux [63]. Lactacidosis is a prominent sequel in ischemic and traumatic brain tissue resulting in glial cell swelling. The swelling mechanism involves activation of coupled Na^+/H^+ and Cl^-/HCO_3^- antiporters, resulting in intracellular accumulation of NaCl and water. There is no evidence for compensatory mechanisms to regulate swelling under these conditions. Also there is as yet only scarce information about activation of osmolyte fluxes associated with this model of swelling. A microdialysis study on NH_4^+ -induced acidosis reports an increase in extracellular N-acetyl-aspartate, an amino acid present in large amounts in neurons, which may have an important role as osmolyte [4].

There is scarce information about possible signalling cascades for activation of mechanisms of cell volume control in isosmotic swelling. The work by Phillis, O'Regan and their collaborators [60] in a model of ischemic rat brain cortex, have shown the influence of PKC based on the stimulatory effect of phorbol esters and the inhibition by chelerythrine on glutamate and aspartate fluxes increased during ischemia. PKA seems not involved in this process. It also documents the importance of phospholipases, PLC and particularly of PLA2, in this mechanism of ischemia-induced amino acid efflux [60]. They also suggest an influence of PTK, since the tyrosine kinase inhibitor genistein, attenuates neurotransmitter release from the ischemic rat cerebral cortex [64]. Recently these authors have made a comparison between the features of amino acid release during ischemia and during hyposmotic swelling in the rat brain. They found important differences, particularly regarding the role of PLA2, which being critical for activation of amino acid release in ischemia, seems to play a minor role in the hyposmotic-stimulated release of amino acids in the same preparation [32]. Also, while PKC modulates ischemia-induced amino acid release, it has no major influence on the hyposmolarity-associated release [32]. These comparative studies are crucial to identify the signalling elements associated with isosmotic swelling within the complex set of responses evoked by ischemia.

Identification of the transduction cascades in isosmotic swelling may be further complicated by the fact that essentially all conditions generating this type of swelling represent severe stressful situations, resulting in activation of numerous signalling elements associated with stress [65]. Some of them such as MAPK and PI3K, are also activated during hyposmotic swelling. The associated events of depolarization and excitotoxicity specific to brain tissue, also activate numerous signalling cascades [66]. All this makes it very difficult to discriminate among the spectrum of responses, those solely attributable to swelling. An experimental maneuver to circumvent this problem, which could be approached in preparations *in vitro*, is to elicit the ischemic or any other situation of cytotoxic swelling, but reducing the external concentration of Cl^-, which would largely attenuate swelling without decreasing the depolarization or other stimuli. Then, a parallel analysis of the signalling cascades activated in both conditions, may help to identify those associated with swelling, and from them, those related to the corrective fluxes of osmolytes.

It has been consistently observed that cytotoxic edema *in vivo* is more prominent in astrocytes than in neurons, being so far unclear whether this difference is due to a selective localization of the swelling-generating mechanisms in astrocytes, or to the presence in neurons of more efficient mechanisms of cell volume control. In this respect, a most interesting observation is the transfer of taurine and glutamate from neurons to astrocytes during experimental ischemia [67]. By this mechanism, neurons are spared and protected from the deleterious effects of swelling.

Isovolumetric regulation: role of amino acids

Although the experimental model of sudden and marked decreases in osmolarity has rendered valuable information to elucidate the 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 resistance. A paradigm closer to the situation in vivo, was that assayed first by Lohr and Grantham [68] in renal proximal tubules, in which cells were exposed to small and gradual decreases in osmolarity. Under these conditions, cell volume remains stable over a broad range of osmolarities, even when the osmolarity drops up to 50%. This constancy in cell volume was named "isovolumetric regulation" (IVR). This term has implicit the idea of an active mechanism of cell adjustment, based on the shrinkage in cells returned to an isosmotic medium, which due to the loss of intracellular osmolytes is now hyperosmotic with respect to the intracellular medium [68]. After this early report, IVR has been observed in the renal cell line A6, in the glioma cells C6 [69, 70], in cerebellar granule neurons (unpublished), in cardiomyocytes [71] and in a more integrated preparation, the hippocampal slice [72]. In contrast, partial IVR is found in cardiomyocytes and no IVR is observed in trout erythrocytes [71, 73] . The mechanisms subserving IVR have not been explored in detail. In A6 cells, increased K^+ efflux is observed at 30% reductions in external osmolarity [68]. K^+ efflux with a similar threshold was found in cultured cerebellar granule neurons (unpublished results) and in cardiomyocytes [71]. K^+ efflux during IVR is decreased by Ba^{2+}, but is insensitive to 4AP, TEA and charybdotoxin (unpublished results). The Cl^- pathway activated during IVR seems to have marked differences with the VSCC, as IVR is impaired when Cl^- is replaced by other anions which permeate through VSCC [69]. This may suggest the involvement of electroneutral cotransporters, known to be more selective for anions than VSCC. In hippocampal slices, IVR occurred without any measurable release of K^+ [72]. This is an unexpected finding which may be attributable to the large K^+ buffering capacity of astrocytes. We have addressed the role of amino acids in IVR in hippocampal slices and found increased fluxes of taurine, GABA and glutamate (Fig. 2). The efflux of taurine shows the lowest threshold, and the highest efflux rate with 4-10-fold differences at essentially all osmolarities. This may reflect a higher permeation through the efflux pathway or/and more availability of the taurine pools to be released in response to the change in cell volume. This may be related to features of taurine such as its metabolic inercy and its mainly cytoplasmic location, while GABA, glycine and glutamate, which have a prominent role as synaptic transmitters or are part of numerous metabolic cascades, may be sequestered in compartments which restrict their availability for osmosensitive release. Taurine efflux during IVR has been shown in cardiac myocytes and in trout erythrocytes, with reductions of 10-17% in taurine cell content [71, 73].

About 30% in average, of the amino acid content in cells or slices is released during IVR. This is clearly insufficient to compensate for the change in external osmolarity when K^+ fluxes have not yet been activated. Therefore, other factors should be considered to explain the maintenance of cell volume under these conditions. One or several of the following possibilities are plausible: 1) swelling is overall restricted when the osmolarity change is small and gradual, 2) other organic osmolytes, such as creatine, myo-inositol, sorbitol, N-acetyl aspartate, phosphocreatine and phosphoethanol amine, are also contributing to counteract the external osmolarity, and altogether compensate for the initial phase of hyposmotic stress, 3) a Cl^- efflux activates, accompanied by cations other than K^+, 4) 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 [74]. In more integrated preparations, such as the hippocampal slice, it may happen that swelling occurs in some but not in all cells, and therefore, the decrease in amino acids and other osmolytes is required to compensate the change in cell volume only in a minor population of cells. Also, a redistribution of osmolyte amino acids between different types of cells i.e. neurons and astrocytes may occur, as observed in mice cerebellum where taurine is translocated from Purkinje cells to astrocytes in response to hyponatremia [75]. In this situation, even though amino acids contribute importantly to regulate cell volume in specific types of cells, this may not result in a large net efflux. Finally, it should be no-

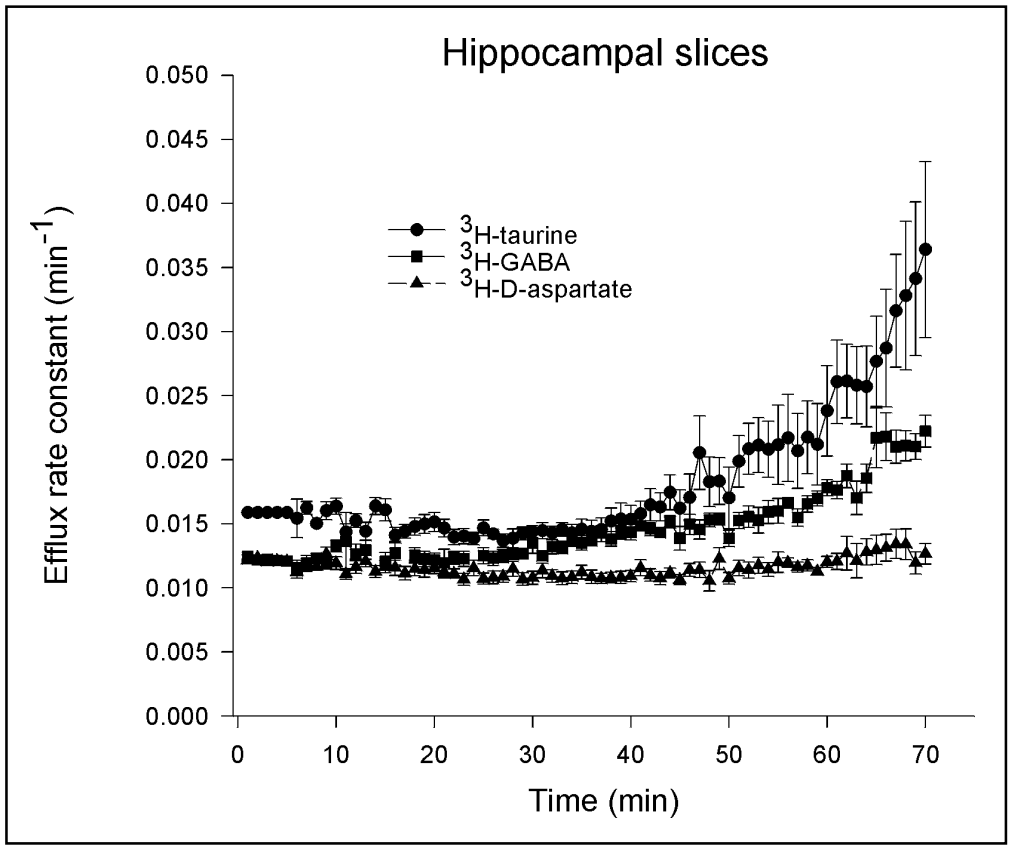

Fig. 2. Amino acid release from hippocampal slices exposed to gradual and progessive reductions in external osmolarity. Slices preloaded with [^{3}H]-taurine (●), D-[^{3}H]aspartate (■), or [^{3}H]GABA (▲), were superfused 10 min with isosmotic medium. At the time pointed by the arrow the external osmolarity was continuosly decreased at a rate of –2.5 mOsm/min until the medium osmolarity reached 150 mOsm (50% hyposmotic). Data are expresed as efflux rate constant (min^{-1}) and are means ± SE (n = 8-10).

ticed that in pathological situations, such as chronic hyponatremia, when the external osmolarity decrease is small but persists during several hours, or even days, the decrease in amino acids and other osmolytes during this period is substantial, being almost 90% in the case of taurine [2]. This has been reproduced in vitro by Olson [76] in cultured astrocytes, which showed no change in cell volume after 24 h of hyposmolarity, coincident with an almost total depletion of the taurine pool, and no significant changes in the concentration of glutamate and K^+. This points to the role played by taurine as an osmolyte of choice for cell volume control in physiopathological conditions.

The similarities or differences, which may exist between the amino acid osmolyte pathway activated during IVR and RVD, have not been explored in detail. There is also no information about transduction signalling cascades leading to activation of this mechanism of cell volume control. In this respect, it is worthy to mention that IVR may be a better system than RVD for the study of signalling cascades primarily associated with osmotransduction. In the absence of the dramatic changes in cell volume occurring during RVD, changes associated with cytoskeleton reorganization, adhesion and even stress, would be reduced and consequently, the remaining set of signals expressed during IVR may be more easily adscribed to specific aspects of cell volume regulation.

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