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Osmosensitive Taurine Release: Does Taurine Share the Same Efflux Pathway with Chloride and Other Amino Acid Osmolytes?

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1. Introduction

Swelling subsequent to hyposmotic conditions activates a process of volume regulation present in most cell types. This volume adjustment is accomplished by osmolyte translocation toward the extracellular space to reach a new osmotic equilibrium. Molecules involved in this homeostatic mechanism have been broadly classified into two categories: organic and inorganic osmolytes. Inorganic osmolytes comprise mainly the intracellular ions K^+ and Cl^-. Cell swelling–induced activation of separate K^+ and Cl^- channels has been described in most preparations. Organic osmolytes are grouped in three categories: amino acids, polyalcohols, and methylamines. These osmolytes, particularly taurine, are present in high intracellular concentrations and may also play a role as cytoprotectants.[1] Amino acids are part of the organic osmolyte pool contributing to RVD in most cells.[1,2] Among them, taurine has been studied in detail mainly because of its metabolic inertness, and it is often considered as representative of all osmolyte amino acids.

1.1. Volume Sensitive Organic Osmolyte and Anion Pathway

Volume regulatory loss of organic osmolytes has been characterized in a wide range of cell types. Efflux of these osmolytes seems to be mediated by passive concentration gradient-driven pathways, which do not exhibit saturation in their efflux profile and are not susceptible to trans-stimulation.[3] It is now generally accepted that swelling-activated organic

osmolyte release is achieved by diffusion through membrane pores, rather than by carrier transport. Transport pathways for organic osmolytes are in general Na^+-independent and nonstereoselective. A particular feature of organic osmolyte release is its sensitivity to general anion channel blockers.[4–6] This has raised the question of whether the volume-sensitive Cl^- channel may be the permeation pathway for these osmolytes. Electrophysiological evidence has shown that amino acids and some other organic osmolytes permeate through the volume-sensitive anion/Cl^- channel (VSAC), which has broad permeability, and the necessary size pore (8–9 Å)[2,7] to allow translocation of amino acids as large as glutamate, as well as other structurally unrelated osmolytes. This channel was named volume-sensitive organic osmolyte/anion channel (VSOAC) by Strange and coworkers.[8] Experimental evidence for the existence of this common pathway is not conclusive so far, and as the characterization of the volume-sensitive Cl^- channel and the organic osmolyte fluxes progresses, evidence against the notion of a common Cl^-/osmolyte pathway becomes less consistent. In this review, we address basic questions that remain unanswered: (1) may the volume-sensitive Cl^- channel be a common pathway for both Cl^- and taurine?; and (2) are taurine and other amino acid osmolytes translocated through the same efflux pathway? We present here results pertaining to these two possibilities and discuss the current state of the field.

2. Evidence against a Common Pathway for Swelling-Activated Taurine and Cl^- Release

Taurine is found at concentrations of up to tens of millimolar in vertebrate cells under physiological conditions. It has a pK2 of 8.82 and is therefore present in the cytosol predominantly as an electroneutral zwitterion.[9] Swelling-activated taurine release (SATR) has been observed in almost all preparations studied to date. SATR occurs via a nonsaturable Na+-independent transport pathway, inhibited by both anion exchanger blockers (DIDS, SITS, niflumic acid, and pyridoxal phosphate), and chloride channel blockers (NPPB, dideoxyforskolin and tamoxifen), and modulated by tyrosine kinases and PI3K activity,[for rev. see 10 and 11] as has been also reported for VSAC.[12–14]

There is recent evidence against a common pathway for both osmolytes. In Ehlrich ascites cells SATR and VSAC fluxes are pharmacological distinct,[15] the former being inhibited by DIDS and niflumic acid, and stimulated by arachidonic acid and LTD4, while the later is inhibited by arachidonic acid, tamoxifen and insensitive to DIDS and niflumic acid.[16] Studies in rat mammary gland and skate erythrocytes demonstrate SATR release without an activation of VSAC,[17–20] and the opposite was observed in human biliary cell line.[21] In skate erythrocytes, the osmosensitive taurine and Cl^- fluxes appear mediated by different pathways, taurine through a channel and Cl^- by cotransporter pathways.[20] Different pathways for SATR and VSAC were also suggested in HeLa cells,[22] where SATR was more sensitive to DIDS than I^{125} efflux. In this study SATR and I^{125} efflux elicited differences in their kinetic activation and inactivation profiles. Differences between VSAC and SATR in their sensitivity to Cl^- channel blockers have been found in NIH3T3 and CHO cells.[23,24] In NIH3T3 cells, the small G-protein Rho A modulates the Cl^- channel conductance while SATR remains almost unaffected.[25] SATR, but not swelling-activated Cl^- conductance, occurs in Xenopus laevis oocytes.[26]

3. The Swelling-Activated Taurine and Amino Acid Release

Taurine is convenient for studies aimed to characterize osmolyte fluxes because it is abundant in cells and tissues and is essentially metabolically inert, and has been often considered as representative of amino acids and other organic osmolytes. Besides taurine, other amino acids translocate in response to hyposmotic swelling. In the same way, glycine, β-alanine, GABA, leucine, glutamine, aspartate, and glutamate permeate through the swelling-activated Cl^-/anion pathway with Paa/Pel ranging from 0.25 to 0.78 in cell types including MDCK cells, inner medullary collecting duct IMCD cells and glial cells.[27–31] Amino acid release as for the case of taurine is inhibited by Cl^- channel blockers (SITS, DIDS, L644-711, niflumic acid, NPPB, dideoxyforskolin and tamoxifen, furosemide, 9-AC, and dipyridamole) in different preparations including primary cortical and cerebellar astrocytes, cerebellar granule neurons and cultured cortical neurons, neuroblastoma CHP-100, cortex, hippocampal slices, chick retina, NIH3T3 cell, and endothelial cells.[23,32–31] Hyposmotic-induced N-acetylaspartate release has been studied in rat striatum preparations, hippocampal slices *and in vivo* studies; similar to SATR, N-acetylaspartate release was also inhibited by Cl^- channel blockers.[40, 43–46] These results may suggest a common efflux pathway for SATR and other amino acids and would, in principle, allow the extrapolation of SATR results to all other osmolyte amino acids.

Recent results from our and other laboratories indicate that the osmosensitive taurine efflux properties clearly differ from those of amino acids such as GABA and glutamate which may act as neurotransmitters. The efflux rate of taurine, glutamate, and D-aspartate were similar in cultured astrocytes regarding the time course and the effect of Cl-channel inhibitors, but are differentially modulated by tyrosine kinase blockers.[34–37,47] More important differences between SATR and other amino acids were found in hippocampal[39, 48] and cortex slices (fig. 1), including (1) The osmosensitive amino acid neurotransmitter release shows a kinetic release profile notably different from SATR. Taurine efflux exhibits delayed activation and inactivation while that of glutamate and GABA fully activate immediately after the stimulus and also rapidly inactivate, (2) GABA and glutamate efflux are insensitive to Cl^- channel blockers, which typically inhibit SATR, (3) SATR is modulated by signaling cascades involving tyrosine phosphorylation events, including an important role for the tyrosine kinase target PI3K; while GABA and glutamate fluxes are not responsive to tyrosine phosphorylation state nor to the influence of PI3K activity, and (4) swelling-activated neurotransmitter release, but not SATR, is influenced by the cytoskeleton depolymerization and manipulation of PKC activity.

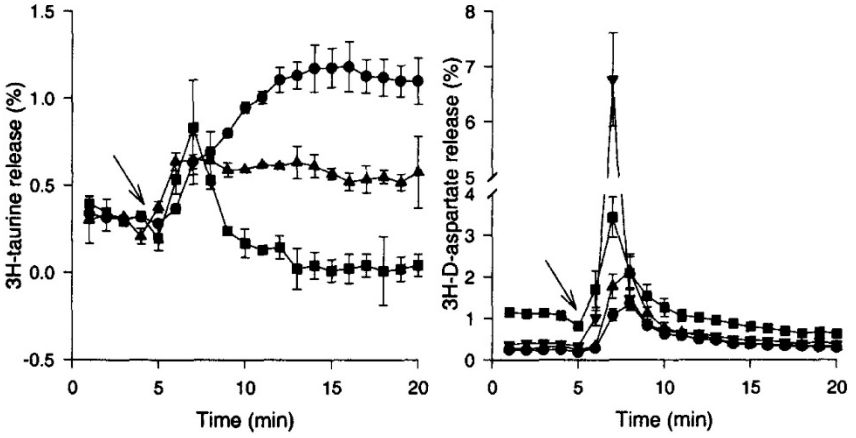

Figure 1. Amino acid release from cortex slices exposed to 30% hyposmotic medium. Slices preloaded with ^{3}H-taurine or ^{3}H-D-aspartate were superfused *5* min with isosmotic medium. Thereafter (arrow), the medium was replaced by 30% hyposmotic solution. One-min fractions were collected during 20 min. ^{3}H-Taurine release was inhibited by tyrosine kinase blockers (50μM AG18 (■))and PI3K inhibitors (wortmannin l00nM (▲)). In contrast, glutamate release (followed as ^{3}H-D-aspartate) was insensitive to these agents but potentiated by PKC activation (l00nM PMA (▼)). (●) Controls with vehicle. Data represent the radioactivity released per min expressed as percentage of the total incorporated and are means ± SE (n = 4–6).

In the same way, in rat cerebral cortex dialysates and bullfrog sympathetic ganglia different effects were obtained between SATR and SAAAC in the presence of several Cl^- channel blockers, SATR being the most widely sensitive and that of GABA, D-aspartate, and glutamate rather inhibited in the presence of these agents.[49,50] Moreover, *in vivo* studies show that glutamate and aspartate are preferentially released during acute hyponatremia while taurine release is sensitive to both chronic and acute hyponatremia.[51] In cultured neurons taurine efflux is more sensitive to osmolarity perturbations than glutamate and GABA.[52] Hyposmotic-induced amino acid neurotransmitter release in excitable cells has been suggested to involve an exocytotic mechanism elicited in response to cell depolarization by either Cl^- release or by activation of nonselective cation channels; or by calcium-independent vesicle fusion mechanisms.[reviewed in 53]

4. Concluding Remarks

The molecular identification and characterization of the swelling-activated efflux pathway for organic osmolytes release has been attempted by several research groups in the field. During their efforts there was initial evidence that suggested a common efflux pathway named VSOAC for both swelling activated-organic osmolyte release and VSAC. This led to the common extrapolation of experimental results from one osmolyte release (either taurine or chloride) to others (like amines, amino acids, or polyalcohols). In this review, we

summarize increasing evidence that points to different efflux pathways for SATR with respect to the VSAC. We also show that for the organic osmolyte group of amino acids, care must be taken in the further extrapolation of SATR to other swelling-activated release of amino acids at least for the case of neurotransmitter amino acids in excitable cells. In this case, it is clear that in brain preparations, different pathways mediate the release of amino acid neurotransmitter release (like GABA and glutamate) with respect to that of SATR. In brain preparations, the different regulatory mechanisms and efflux pathways involved in the swelling-activated amino acid neurotransmitter release with respect to that of taurine may be of physiological relevance because high extracellular concentrations of these neurotransmitters may lead to exocytotic insults, during conditions of cell swelling. It must be taken into account that several efflux pathways may be activated during cell swelling conditions for different osmolytes or even for the same one.[54–56] All this data challenge the hypothesis of VSOAC as the common efflux pathway for the release of organic osmolytes and anion conductance elicited by cell swelling conditions, and even for a common pathway for taurine and other amino acid osmolytes in excitable cells.

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