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Influence of protein tyrosine kinases on cell volume change-induced taurine release

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Abstract
Taurine efflux occurs in association with cell swelling in both hyposmotic and isosmotic conditions and during cell shrinkage in apoptotic death. Release occurs through a leak pathway, is largely Ca^{2+}-independent and is sensitive to Cl^- channel blockers. Taurine efflux elicited by hyposmolarity is reduced or suppressed by tyrosine kinase blockers and increased by tyrosine phosphatase inhibitors. The specific kinases involved are still unknown and may be different in the various cell types. Non-receptor and scr-related protein kinases have been identified in some cells as elements that directly phosphorylate the taurine efflux pathway. Possible tyrosine kinase targets are the phosphinositide kinase (PI3K), which if inhibited, prevents the osmosensitive taurine efflux in brain cells, or the small GTP-binding proteins associated with remodeling of the cytoskeleton. The similar effects of tyrosine kinase modulators on volume-activated taurine fluxes and Cl^- currents are suggestive of either a shared translocation pathway or a common step in the signaling network. The effects of tyrosine kinases on taurine efflux activated in isosmotic swelling and in the release associated with apoptosis are essentially unexplored.

Keywords: PI3 kinase, swelling, hyposmolarity, osmolytes

Introduction

Taurine is released from diverse brain tissue preparations in response to a variety of stimuli, one of the most conspicuous being the reduction in external osmolarity.[1] Taurine release is activated by cell membrane depolarization and in conditions of ischemia, hypoxia,

hyperammonemia and subsequent to generation of oxygen free radicals and membrane lipid peroxidation.[2–5] Another stimulus consistently increasing taurine release from brain preparations is the activation of excitatory amino acid receptors by agonists such as glutamate, kainate, NMDA, and quinolinic acid. Nitric oxide has been reported to increase taurine efflux from cultured neurons and brain slices, an effect that could be either direct or through modulation of NMDA receptors.[2] It is so far unclear whether the release of taurine in all these different conditions is triggered by a common factor, or whether in each case the release operates through different avenues. A possibility is that taurine release is primarily a cell response to swelling. The bases to consider this possibility are, first, that all these conditions involve a certain degree of swelling and second, that swelling consistently evokes taurine efflux. Taurine is particularly suited for this osmolyte role, due to its metabolic inactivity and its high concentration as a free solute in the cytosol.[6]

Taurine efflux is evoked by hyposmotic and isosmotic cell swelling

Swelling of brain cells may occur in hyposmotic or isosmotic conditions. The first condition is the consequence of hyponatremia of various origins.[7] Isosmotic cell swelling, also referred as cytotoxic edema, occurs in association with ischemia, epilepsies, hepatic encephalopathy, hyperammonemia, head trauma, and hypoglycemia.[8–11] Swelling results from changes in ion distribution by dysfunction of ion transport mechanisms or by disruption of the membrane barrier subsequent to attack by free radical and lipoperoxidation, leading to ionic imbalance and overload, with the consequent water accumulation.[8–11] In all cases, brain cell edema represents a situation of high risk, since the restriction to tissue expansion imposed by the rigid cranium, commonly leads to compression of small vessels generating episodes of ischemia, excitotoxicity, and neuronal death.

Experimental paradigms *in vitro* and *in vivo* have been designed to simulate the conditions leading to brain cell edema *in vivo*. The most commonly used model for hyponatremia is to expose cells or tissue to a hyposmotic medium made by decreasing the concentration of NaCl. Under these conditions, cells initially swell and then undergo an active process of volume recovery accomplished by the extrusion of intracellular osmolytes. Hyposmolarity elicits taurine efflux in essentially all cell types, including brain cells and also in brain tissue preparations.[1,12,13] *In vivo*, taurine efflux from brain superfused with hyposmotic medium has been also consistently observed, and the increase of taurine in the extracellular space mirrors the decrease in intracellular levels.[14,15,16] The magnitude of taurine efflux is proportional to the reduction in osmolarity, its time course roughly parallels that of the volume recovery and blockade of taurine efflux or taurine deficiency impairs cell volume regulation.[1] In models of chronic hyponatremia *in vivo*, a continuous and progressive decrease in brain taurine levels is observed, to an extent that the intracellular pool is essentially depleted.[17] These results underlie the importance of taurine in the maintenance of normal brain water content, preventing the sequels of injury due to cell edema.

There is general agreement that osmosensitive taurine efflux occurs through a leak pathway, with no contribution of the Na^+-dependent carriers.[18] In most cell types taurine efflux is sensitive to Cl^- channel blockers, such as 5-nitro-(3-phenyl-propylamino) benzoic acid

(NPPB), tamoxifen, and to the anion exchanger blockers niflumic acid, 4,41-diisothiocyanatostil loene-2, 21-discultonic acid (DIDS) and pyridoxal phosphate.[19–21] These features have raised the proposal of a common pathway (a Cl^- channel-like molecule), for the osmosensitive transport of Cl^-, taurine, and other organic osmolytes.[19] This common pathway hypothesis is not yet conclusive[21] and awaits the molecular identity of the volume-sensitive Cl^- channel as well as more insight into the molecular nature of the taurine pathway itself.

While cell volume regulation after swelling evoked by hyposmolarity has been extensively investigated, it is still unclear whether such regulation occurs in cytotoxic edema and to what extent organic osmolytes may participate in the volume correction, or at least contribute to reduce the magnitude of swelling. Taurine release occurs consistently under conditions of cytotoxic edema[2] and its release is sensitive to Cl^- channel blockers, suggesting a similarity with the mechanism of release in hyposmotic conditions.[22–24] However, it should be emphasized that even when cell swelling occurs by different mechanisms in each pathology, in most of them Cl^- influx is a causal factor. Therefore, the inhibitory action of Cl^- channel blockers may be exerted on the Cl^- influx pathways, thus preventing swelling and consequently, the primary cause of taurine release.

Tyrosine kinases are involved in the hyposmolarity-evoked release of taurine

Most studies aiming to understand the influence of tyrosine kinase-activated pathways during volume correction after hyposmotic swelling have been focused on the volume-activated Cl^- channel,[25] while similar investigations for taurine efflux are still scarce. As mentioned before, the one pathway hypothesis for Cl^- and taurine is not yet conclusive and therefore, results about signaling mechanisms modulating volume-activated Cl^- channels should not be directly extrapolated to the osmosensitive taurine efflux. Similarly, transduction steps leading to activation of taurine efflux should not be naturally assumed to be the same for other organic osmolytes, nor even for other amino acids. This may be particularly true for tyrosine kinases as will be later discussed.

Evidence in support of the involvement of tyrosine kinases in the osmosensitive taurine efflux relies on the effect of several tyrosine kinase blockers reducing the efflux of taurine, while correspondingly, agents which inhibit tyrosine kinase phosphatases and prolong tyrosine phosphorylation, potentiate taurine release.

The general tyrosine kinase blockers tyrphostins, inhibit taurine release evoked by swelling in cultured cerebellar granule neurons and astrocytes,[26,27] in the supraoptic nucleus[28] and in hippocampal slices[29] (table 1). Ortho-vanadate potentiates taurine efflux in cerebellar granule neurons and astrocytes and in the supraoptic nucleus whereas it has no effect on taurine efflux in hippocampal slices. These differences, also found in other cells (see below) may be due either to the involvement of tyrosine phosphatases with different sensitivities to the blocker or to differences in the permeability of ortho-vanadate in the various preparations.

Table 1. Effect of tyrosine kinase blockers in hyposmotic-induced taurine efflux in brain preparations

Cell type	Agent	[μM]	Effect	Reference
Cerebellar granule neurons	Tyrphostin AG18	50	Inhibition 90%	(26)
	Genistein	50	Inhibition 30%	
	PD98059	25	Unaffected	
Cerebellar astrocytes	Tyrphostin A23	0.32–320	Inhibition 90%	(27)
	Tyrphostin A51	0.1–100	Inhibition 50–55%	
Supraoptic nucleus	Tyrphostin B44	50	Inhibition 27–32%	(28)
	Genistein	50	Inhibition	
	PD98059	50	Unaffected	
Hippocampal slices	Tyrphostin AG18	50	Inhibition 61%	(29)
	Tyrphostin AG112	100	Inhibition 29%	
	Tyrphostin AG879	25	Inhibition 78%	
	Genistein	100	Unaffected	
	Lavendustin A	1	Unaffected	
	Herbimycin A	10	Unaffected	
	PD98059	25	Unaffected	
	SB202190	25	Unaffected	

Tyrosine kinase blockers also reduce the hyposmolarity-elicited taurine efflux in non-brain cells (table 2). In skate blood erythrocytes, tyrphostins reduce hyposmolarity-induced taurine release[30] and in HeLa cells, genistein decreases and ortho-vanadate potentiates taurine efflux. Also genistein reduces taurine efflux in trout erythrocytes, but in the latter cells, the release is either unaffected or reduced by ortho-vanadate.[32] In a Langendorff heart preparation, taurine efflux is reduced by genistein and lavendustin A.[33] Remarkably, this is the only study *in vivo*, addressing the influence of tyrosine kinases on the mechanism of taurine release in hyposmotic conditions.

Table 2. Effect of tyrosine kinase blockers in hyposmotic-induced taurine efflux in nonbrain preparations

Cell type	Agent	[μM]	Effect	Reference
Skate red blood cells	Tyrphostin 46	25–200	Inhibition	(30)
	Tyrphostin A23	25–200	Inhibition 50%	
	Genistein	25–200	Stimulation <20%	
	PD98059	25–200	Stimulation <20%	
Trout red blood cells	Genistein	45–100	Inhibition	(32)
Jurkat T-cells	Herbimycin A	10	Unaffected	(53)
HeLa cells	Genistein	100	Inhibition 60%*	(31)
Langendorff preparation	Genistein	1	Inhibition	(33)
	Lavendustin A	0.5	Inhibition	

* Recalculated from (31)

While taurine efflux stimulated by hyposmolarity is clearly affected by tyrosine kinase blockers, other amino acids (GABA and glutamate) which are also released from brain cells by the hyposmotic stimulus, are insensitive to tyrosine kinase blockers, suggesting a lack of influence of these kinases.[29] A similar insensitivity to Cl^- channel blockers, in contrast to taurine, suggests a different pathway for the osmosensitive release of these amino acids.[29]

In most cell types, the specific kinases involved in the osmosensitive taurine efflux have not yet been identified

The consistent effect of tyrosine kinase blockers reducing taurine efflux in hyposmotic conditions, point to these enzymes as part of the osmotransduction signaling, connecting the change in cell volume and the activation of the taurine efflux pathway. So far, though, in most cells the specific kinases involved and the sites of their action remain to be identified. The most consistent work on the involvement of tyrosine kinases in hyposmolarity-activated taurine release has been carried out by Goldstein and coworkers, in a series of studies in the skate nucleated erythrocytes. The hyposmolarity-induced release of taurine in these cells appears to occur through the band 3 anion exchange protein.[34] Taurine release is reduced by tyrphostin A23, although it is insensitive to other tyrphostins and to genistein.[30] Hyposmosis leads to band 3 phosphorylation by the tyrosine kinases p76syk and p56lyn[30] and p72syk directly phosphorylates the cytoplasmic domain of the band 3 protein with a time course parallel to that of the swelling-activated taurine efflux.[30] Accordingly, the p72syk inhibitor piceatannol blocks, at about the same concentration, both taurine efflux and p72syk-dependent band 3 phosphorylation.[30] Moreover, the phosphatase blocker pervanadate similarly potentiates taurine efflux and the activity of p72syk phosphorylation.[30] In this same preparation, various experimental paradigms were used to produce cell swelling with differences in the intracellular ionic strength, as this parameter seems to regulate the set point for taurine efflux activation by cell swelling.[35,36] The effect of intracellular strength reduction on the activity of the two tyrosine kinases involved in the band 3 phosphorylation and taurine efflux was examined, and results are suggestive of some sensitivity of p72syk and p56lyn to the change in intracellular ionic strength.[37] In this respect, in the supraoptic nucleus, the magnitude of the effect of tyrosine kinase and phosphatase blockers is dependent on the external osmolarity, suggesting that tyrosine kinases modulate the sensitivity to hyposmolarity rather than the efflux pathway activation.[28]

The src-related tyrosine kinase p56lck is found to be a key signaling element for osmosensitive Cl^- currents in lymphocytes, as shown by its activation upon swelling, suppression of osmosensitive currents in p56lck-deficient cells by genetic knockout and its restitution after retransfection of the kinase.[38] The involvement of this kinase on taurine efflux has not yet been examined.

Apart from these studies in blood cells, the tyrosine kinases involved in taurine efflux, and in that of other osmolytes as well, have not been identified. This is further complicated because tyrosine kinases are steps in the signaling cascades of numerous cell responses, many of which are activated by processes occurring in association with swelling and volume regulation, such as cell adhesion and retraction, reorganization of the cytoskeleton, and gating of stretch-activated channels. Besides, swelling is a stressful condition for the

cells, also activating signals associated with stress responses. Therefore, even the identification of the kinases activated by hyposmolarity (fig. 1) may not be sufficient to ascribe them to the activation or operation of osmolyte extrusion pathways, including those for taurine. Examples of this situation are the phosphorylation by hyposmotic swelling of the mitogen-activated protein (MAP) kinases extracellular signal-related kinase-1/2 (ERK1/ERK2) observed in various cell types.[25,39] This reaction mediates the swelling-induced transcription of early expression genes,[40] the volume-sensitive channels in astrocytes[41] and taurocholate excretion in hepatocytes;[39] but in most cell types and brain preparations such as cerebellar granule neurons, hippocampal slices, supraoptic nucleus, intestinal 407 cells and skate erythrocytes, even when MAP kinases are activated by hyposmolarity, preventing this reaction has no effect on osmolyte fluxes, including Cl^- and taurine.[25,26,28,29,42]

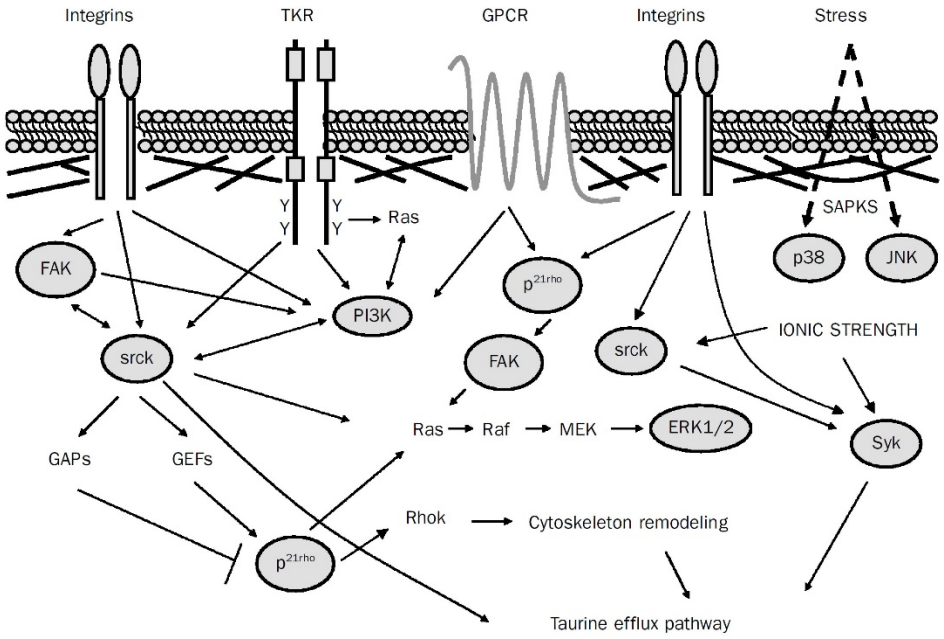

Figure 1. Interactions between some membrane associated receptors and signaling cascades involving tyrosine kinases and tyrosine kinase-activated kinases. Enzymes in shaded circles are those activated by hyposmolarity. Receptors may be activated by adhesion reactions or by clustering due to membrane cytoskeleton reorganization as a consequence of swelling. TKR: tyrosine kinase receptors, GPCR: G protein coupled receptors, FAK: focal adhesion kinase, PI3K: phoshoinositide 3-kinase, SAPKS: stress-activated kinases, ERK1/2: extracellular regulated kinases 1 and 2, MEK: MAPK/ERK-kinase, GEFs: guanine exchange factors, GAPs: GTPase-activating proteins, srck: src-related kinases, Rhok: Rho-kinase.

An attractive hypothesis recently considered is that the integrin receptor could play a role in the sensing of cell volume changes by detecting membrane expansion or unfolding of membrane invaginations.[43] Integrin activation, possibly by receptor clustering, could initiate a signaling cascade via focal adhesion kinase (FAK), which is known to be activated

by hyposmolarity. FAK, in turn, connects with a variety of signaling proteins such as src-related protein kinases, PI3K and small GTP-binding proteins associated with remodeling of the cytoskeleton. All these proteins have been found to be activated by hyposmolarity in different cells and many of them are related to taurine (or Cl^-) corrective fluxes. Besides the already discussed link of taurine release with src-related kinases and other nonreceptor kinases in blood cells, other elements of this signaling cascade involved in osmolyte fluxes include the small GTPase p21Rho,[44,45] and particularly the phosphoinositide kinase PI3K. This enzyme, which is a key element in signaling networks connecting with integrins, FAK, src-related kinases and tyrosine kinase membrane receptors, appears to have a relevant role in the mechanisms of taurine release. In cerebellar granule neurons, astrocytes, and hippocampal slices, hyposmolarity activates PI3K, and this reaction is blocked by wortmannin (fig. 2A).[26,29] This agent is also a potent blocker of hyposmotic taurine release in these preparations (fig. 2B), suggesting a direct link of PI3K and the activation or/and operation of the taurine efflux pathway. Wortmannin also blocks anion fluxes activated by hyposmolarity in various cell types.[44,47,48] The similar effect of tyrphostins and wortmannin on taurine efflux illustrated in figure 2 is suggestive of PI3K as a main target of tyrosine kinase reactions in osmotransduction.

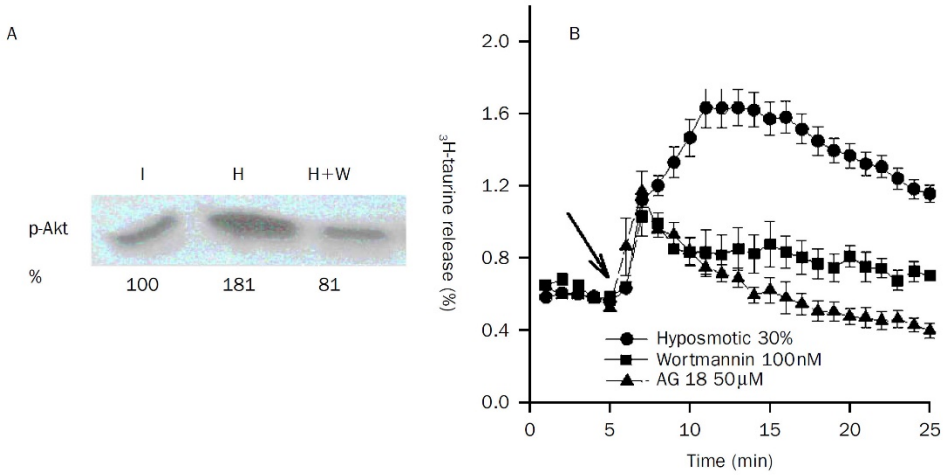

Figure 2. Effect of tyrosine kinases and PI3K inhibition on the osmosensitive taurine efflux hippocampal slices. (A) Hyposmotic (30%) medium activates PI3K, sensitive to wortmannin. I: isosmotic, H: 30%, hyposmotic, H1W: 30% hyposmotic + 100 nM wortmannin. Representative results of three independent experiments are shown. PI3K was quantified by detection of the phosphorylated form of Akt phosphorylated at Ser-473. Lower panel shows the % of activity respectively to the isosmotic condition. (B) Inhibitory effect of 50 µM tyrphostin AG18 (s) and 100 nM wortmannin (n) on taurine efflux elicited by 30% hyposmotic medium (l) (arrow) from hippocampal slices preloaded with 3H-taurine. Data represent the radioactivity released per min expressed as percentage of the total incorporated and are means ± SE (n = 6). Details of the experimental conditions as in Ref. 29.

Tyrosine kinase receptors such as integrin, associated with many of those signaling elements may also have an influence on osmolyte release, as suggested by a study on myoblasts, in which taurine efflux is activated by thrombin and hyposmolarity, with similar characteristics.[46] The idea of integrin or other membrane receptors acting as a very early and basic mechanism of the signaling osmotransduction cascade, might also explain the many common effects of tyrosine kinases on Cl^- and taurine fluxes, and possibly on other organic osmolytes as well.

Are tyrosine kinases involved in the mechanism of taurine release in isosmotic conditions?

As previously mentioned, taurine efflux is elicited in isosmotic conditions by a variety of stimuli, most of them having in common some extent of cell swelling due to intracellular ion redistribution. The mechanisms of taurine release in these conditions are still poorly defined and may or may not be similar to those activated by hyposmotic swelling. The influence of tyrosine kinases on taurine release is essentially unknown. Only two reports document the inhibitory effect of genistein and lavendustin on taurine release evoked by ischemia-reperfusion in brain and heart.[49,50] It should be noticed that, similar to hyposmolarity, or even more, ischemia evokes a large number of reactions and cell responses, many of them involving tyrosine kinase phosphorylations, thus making it difficult to identify among those activated by the ischemic condition, which are directly related to the mechanism of taurine release.

Taurine release and apoptosis

Cell shrinkage is one of the hallmarks of apoptosis, though the mechanisms leading to this remarkable reduction in cell volume are still poorly understood. Moderate increases in external osmolarity inhibit apoptosis, suggesting an active role of cell volume as part of the signals leading to cell death.[51] Increasing external osmolarity may prevent the loss of osmotic solutes, and cell shrinkage, which may be a determinant in the initiation of the apoptotic events. Taurine appears to be one of those osmolytes. In Jurkat T lymphocytes, apoptosis induced by stimulation of the CD95 receptor, evokes a release of taurine.[52] An apoptosis-associated taurine release has been also found in cerebellar granule neurons.[53] At variance with the osmosensitive release of taurine, the apoptosis-related taurine efflux is insensitive to Cl^- channel blockers.[52,53] The influence of tyrosine kinases is essentially unknown, with only one report in Jurkat cells showing that src-related kinases are not involved, since neither herbimycin A, vanadate nor p56lck deficiency influenced taurine release.[54]

Conclusions

The release of taurine associated with cell volume regulation is well established in conditions of hyposmolarity and tyrosine kinases likely play a critical role in the mechanisms of this release. The present knowledge, however, is still fragmentary, with studies carried out

in different cell types or tissue preparations, different blockers and different experimental approaches, which makes it difficult to identify the specific tyrosine kinases involved in each cell type. As for the release of taurine in isosmotic conditions, the influence of tyrosine kinases is essentially unexplored, as is that of the apoptosis-associated taurine efflux. This is an interesting avenue for future research, which may give important clues about the similarities and dissimilarities of taurine release evoked by different stimuli.

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