Swelling-Induced Taurine Transport: Relationship with Chloride Channels, Anion-Exchangers and Other Swelling-Activated Transport Pathways

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Key Words

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Abstract

Cells have to regulate their volume in order to survive. Moreover, it is now evident that cell volume *per se* and the membrane transport processes which regulate it, comprise an important signalling unit. For example, macromolecular synthesis, apoptosis, cell growth and hormone secretion are all influenced by the cellular hydration state. Therefore, a thorough understanding of volume-activated transport processes could lead to new strategies being developed to control the function and growth of both normal and cancerous cells. Cell swelling stimulates the release of ions such as K^+ and Cl^- together with organic osmolytes, especially the β -amino acid taurine. Despite being the subject of intense research interest, the nature of the volume-activated taurine efflux pathway is still a matter of controversy. On the one hand it has been suggested that osmosensitive taurine efflux utilizes volume-sensitive anion channels whereas on the other it has been proposed that the band 3 anion-exchanger is a swelling-induced taurine

efflux pathway. This article reviews the evidence for and against a role of anion channels and exchangers in osmosensitive taurine transport. Furthermore, the distinct possibility that neither pathway is involved in taurine transport is highlighted. The putative relationship between swelling-induced taurine transport and volume-activated anionic amino acid, α -neutral amino acid and K^+ transport is also examined.

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Introduction

Cell membranes are very permeable to water which means that cell volume will be determined by the osmolality of the extracellular fluid and by the cellular content of osmotically active solutes. Although the volume of some cells (i.e. kidney and intestinal epithelial cells) can be altered as a consequence of being exposed to anisotonic conditions *in vivo*, cell volume perturbations are more often than not the result of a change in the rate of solute transport and/or a change to the rate of oxidative

metabolism [1]. Most cells however, have the capability to regulate their volume following swelling or shrinking [2] and the processes are respectively known as a regulatory volume decrease (RVD) and a regulatory volume increase (RVI).

The membrane transport mechanisms which enable cells to regulate their volume have long been the subject of intense interest. There are two main reasons for this. Firstly, cells have to regulate their volume within relatively narrow limits to survive: failure to do so would result in cell lysis. Secondly, it is apparent that cell volume and the processes which control it, comprise an important signalling mechanism which is involved in the control of cell metabolism, the initiation of apoptosis, the regulation of the cell cycle and hormone secretion [1, 3, 4]. Protein, lipid and glycogen synthesis are all sensitive to changes in the cellular hydration state [5]. In general, the synthesis of macromolecules is increased and decreased by cell swelling and shrinking, respectively. A reduction in cell volume under isosmotic conditions is one of the earliest events in the apoptotic cascade: the decrease in volume appears to be the result of activating transport processes which are normally induced by cell swelling [6]. Volume-activated Cl^- currents associated with a regulatory volume decrease (RVD) play an important role in controlling the growth of nasopharyngeal carcinoma cells [7, 8]. Furthermore volume-activated transport processes are involved in both muscle and glioma cell proliferation [9, 10]. There is good evidence to suggest a role for volume-activated transport mechanisms in the secretion of insulin. It has been shown that glucose uptake into pancreatic cells induces cell swelling which in turn opens anion channels: the subsequent efflux of chloride contributes to the depolarization of the cell membrane and thus insulin release [11].

Cell volume regulation is facilitated by a wide range of membrane transport systems [2, 12]. For example, cell swelling induces the efflux of K^+ and Cl^- via (K^+-Cl^-) cotransport and/or separate K^+ and Cl^- channels whereas cell shrinking activates transport systems such as ($\text{Na}^+-\text{K}^+-\text{Cl}^-$) cotransport and Na^+/H^+ exchange working in parallel with $\text{Cl}^-/\text{HCO}_3^-$ exchange [2]. Organic osmolytes, especially amino acids, also play an important role in regulating cell volume following swelling [13].

Amino acids also make a significant contribution to the total intracellular osmolality and consequently are able to make a significant contribution to cell volume regulation [14]. In this connection, a considerable amount of effort has been invested into studying volume-activated amino acid transport. A thorough knowledge of swelling-induced

taurine efflux and its association with other volume-sensitive transport pathways will help develop new strategies aimed at controlling the metabolism and growth of both normal and cancer cells. Many studies have focused on the properties of volume-activated taurine transport because it is one of the most abundant amino acids within cells [15]. In addition, taurine lends itself to the study of volume-activated amino acid transport because it is relatively inert and thus the interpretation of transport studies, particularly efflux studies, is not confounded by metabolism.

The study of swelling-induced taurine transport has, however, become a controversial and somewhat complicated area. In particular, there is a long-standing debate about the relationship between osmosensitive taurine transport and volume-activated Cl^- channels and the band 3 anion-exchanger (AE1) [13]. This review will focus on four main issues. First, the evidence for and against chloride channels being a conduit for volume-activated taurine transport will be examined. Secondly, the relationship between volume-activated taurine transport and the band 3 anion-exchanger will be reviewed. Thirdly, the evidence pointing to the existence of more than volume-activated taurine efflux pathway will be discussed. Fourthly, the possibility that the volume-activated taurine efflux pathway is permeable to other amino acids and K^+ will be examined. It is not my intention to review the regulation of osmosensitive taurine transport which has been expertly covered in detail elsewhere [16-19]. However, the regulation of volume-activated taurine transport is referred to when it enables conclusions to be drawn about the relationship between swelling-induced taurine transport and other volume-activated transport mechanisms.

General characteristics of volume-activated taurine release

Swelling-induced taurine efflux is a phenomenon exhibited by most cells. Although it will become evident from this article that some properties of osmosensitive taurine transport are not universal there are however, a number of features common to many cell types. Exposing cells to a hyposmotic shock (or any other stimulus which increases cell volume) stimulates taurine transport in a manner which does not require Na^+ : the increase is dependent upon the extent of cell swelling although the relationship between the two parameters is often non-linear [20-24]. This could reflect that higher hyposmotic

challenges induce the activation of a second taurine efflux mechanism and/or recruits another signalling pathway. Volume-activated taurine efflux is independent from the (Na^+ -taurine) cotransport mechanism (i.e. system β ; TauT) [15]. However, guanidineethyl sulphonate (GES), an inhibitor of TauT, stimulates swelling-induced taurine efflux from supraoptic glial cells suggesting that TauT can be involved in the rapid reuptake of taurine under hyposmotic conditions [25].

In common with volume-activated K^+ and Cl^- efflux, the stimulation of taurine efflux by a hyposmotic shock is rapid and occurs within seconds or minutes [21, 26-30] suggesting that the protein already resides in the plasma membrane or possibly in cytoplasmic vesicles which can be readily inserted into the cell membrane [31]. Swelling-induced taurine efflux is sensitive to a number of structurally diverse pharmacological agents (see below). However, none of the inhibitors markedly reduce taurine efflux under isotonic conditions suggesting that the pathway is quiescent when cells are at a 'normal' volume [32-36].

Cell swelling is able to increase the uptake as well as the efflux of taurine. It is apparent that the swelling-induced taurine transport pathway allows the bidirectional movement of the osmolyte whilst achieving a net outward flux [14]. This attribute has allowed the kinetics of volume-activated transport to be examined with relative ease. Importantly, osmosensitive taurine uptake is not saturable with respect to extracellular taurine which was the first convincing evidence in favour of the hypothesis that channels rather than carriers are involved [14, 37-39]. However, it remains to be shown that volume-activated taurine efflux is non-saturable: this would be technically difficult.

There are a number of other characteristics which appear to be common to volume-activated taurine transport. First, the effect of cell swelling on taurine efflux, induced by a hyposmotic shock, can be reversed fully by returning cells to an isosmotic buffer [25, 40]. However, the time taken to fully activate taurine transport is normally much less than the time taken to inactivate the flux suggesting that factors other than, or in addition to, membrane stretch are involved in the signal transduction process. Second, it is evident that repeated osmotic shocks diminish the size of the volume-activated taurine efflux [28, 41]. The reason for this is not entirely clear but may be related to the loss of intracellular osmolytes following each osmotic perturbation. Third, volume-activated taurine transport appears to 'inactivate' in the presence of a prolonged hyposmotic shock [23, 28, 42, 43]: this is

probably a reflection that cells are regulating their volume during the hyposmotic challenge. Fourth, an abrupt osmotic shock increases taurine efflux to a much greater extent than delivering the same osmotic challenge gradually [41, 44]. Fifth, swelling-induced taurine efflux can also be reduced by lowering the temperature of the incubation buffer which is probably a consequence of interfering with signal transduction processes [22, 41, 42].

Does taurine utilize volume-activated Cl^- channels?

Evidence in support of a shared pathway

Early studies appeared to rule out the involvement of carrier-mediated, swelling-induced taurine transport [14, 37-39, 45]. Rather, it was postulated that channels may provide a conduit for volume-activated taurine release [14, 37, 46]. The finding that swelling-induced taurine transport could be inhibited by the stilbene-derivative DIDS prompted the suggestion that the channels could be chloride channels [28, 38, 47]. Many other studies followed which showed that volume-activated taurine transport could be inhibited by other anion channel blockers. The list grew rapidly to include niflumic acid, flufenamic acid, NPPB and tamoxifen. Several studies also found that anion channel inhibitors blocked volume-activated taurine and $\text{Cl}^-(\text{I})$ transport with similar affinity which appeared to strengthen the argument for a common pathway [40, 48]. However, the drugs used to characterize volume-activated taurine transport are non-selective [49]. Indeed, it is possible that the effects of anion channel blockers on volume-sensitive efflux may be indirect especially when used at high concentrations. For example, it has been established that the opening of the swelling-induced taurine efflux pathway is dependent upon intracellular ATP which in turn can be lowered by certain anion channel blockers [50, 51]. Furthermore, Kirk & Kirk [52] have shown that the inhibition of osmosensitive taurine efflux by certain pharmacological agents correlates with inhibition of calmodulin. However, the action of all anion transport inhibitors on taurine efflux cannot be attributed to an effect on calmodulin because volume-activated taurine release is, in many instances, independent from Ca^{2+} [17]. Even today, despite the relative non-specificity of anion channel blockers, pharmacological inhibition of taurine transport is still the most common method used to implicate the participation of anion channels.

The most convincing evidence for volume-activated

taurine transport utilizing anion channels has come from electrophysiological studies. However, at physiological pH, only a small fraction of taurine exists in anionic form making currents difficult to detect. To overcome this problem most experiments have been performed at pH 8.2–8.3 and with high taurine concentrations (180–500 mM). The first of these studies was performed by Banderali & Roy [53] who showed that amino acids including taurine, were able to permeate volume-activated anion channels in MDCK cells. They reported that the taurine to chloride permeability ratio was 0.75. Soon after, Jackson & Strange [21] were able to confirm, again using electrophysiological techniques, that taurine passed through an anion channel in rat C6 glioma cells. In this instance the taurine to chloride permeability ratio was 0.2. Similarly, it has been shown that the $P_{\text{taurine}}/P_{\text{Cl}^-}$ ratio is respectively 0.31 and 0.15 in 9HTEo⁺ human tracheal and rat IMCD cells [48, 54]. It is also apparent that cerebellar granule cells express a volume-sensitive chloride channel which allows taurine to permeate [55]. More recently, it has been demonstrated, using patch-clamp analysis, that the maxi-Cl⁻ channel which resides in the microvillus membrane of the human placenta is permeable to taurine [56]. The maxi-Cl⁻ channel has been implicated in placental cell volume regulation and therefore it has been suggested that taurine and chloride share a swelling-induced pathway in the syncytiotrophoblast [56].

The apparent common pathway has been named VSOAC (volume-sensitive organic osmolyte and anion channel) [57]. Although the electrophysiological studies provide strong evidence in favour of a common pathway for taurine and Cl⁻ it must be borne in mind that the high pH and taurine concentrations employed in the experiments could affect the physical properties of the channels. Therefore, the argument in favour of a common pathway would be strengthened by the demonstration that volume-activated Cl⁻ channels are permeable to taurine under more physiological conditions. In relation to this, it should be borne in mind that there is a distinct possibility that taurine may exit cells as a zwitterion (the most common intracellular form) which may or may not use anion channels.

Evidence in support of separate volume-activated taurine and Cl⁻ channels

Even as support for a shared pathway (i.e. VSOAC) was growing, several reports were published which pointed to the existence of separate volume-activated taurine and Cl⁻ channels. Thus, Lambert & Hoffmann [26] reported that swelling induced taurine and Cl⁻ channels in Ehrlich

ascites tumour cells displayed different properties. For example, the swelling-induced taurine channel could be activated by arachidonic acid whereas the Cl⁻ channel was inhibited. Another piece of evidence against the widespread expression of VSOAC was that a hyposmotic shock could increase taurine efflux from lactating rat mammary tissue explants without a concomitant increase in Cl⁻(I⁻) efflux [27, 58]. The studies by the latter group do not rule out the presence of volume-activated chloride channels in lactating mammary cells per se but appear to rule out a basolateral location given that explants are believed to give a measure of transport across the blood-facing aspect of the mammary epithelium [59]. Similarly, Culliford et al [35] have shown that cell swelling can increase taurine efflux but not I⁻ release from C6 glioma cells. This is in direct contrast to the results of Jackson & Strange [21] who reported the presence of both osmosensitive taurine and Cl⁻ transport in C6 glioma cells. The reason for the discrepancy is not immediately apparent but could be due to the different culture conditions employed. Nevertheless, it appears that swelling-induced taurine and Cl⁻ channels in C6 glioma cells are separate entities. In a similar vein, Stegen et al [60] have shown that under certain experimental conditions, cell swelling is able to stimulate taurine efflux from *Xenopus* oocytes without a concomitant increase in the chloride permeability.

If swelling-induced taurine and Cl⁻ efflux from a cell is via a single pathway then it can be predicted that the effluxes should have similar time-courses. There are several instances in the literature where this does not apply. Simultaneous measurement of osmosensitive taurine and iodide efflux from HeLa cells showed that the time-courses markedly differed, thus, volume-activated iodide release was transient whereas taurine release was sustained [61–63]. A similar situation has been observed in MDA-MB-231 human breast cancer cells [33]. Thus, a hyposmotic shock induced a rapid but transient increase in iodide efflux whereas the volume-activated component of taurine efflux was maintained over a longer period. It is difficult to envisage a situation whereby a common pathway allows the passage of taurine but not Cl⁻(I⁻).

Another prerequisite for the existence of a common pathway is that volume-sensitive taurine and Cl⁻ transport should have a similar stimulation threshold. In this connection, many studies have shown that osmosensitive taurine and Cl⁻ do have a similar osmotic profile (e.g. see [64]). However, Ordaz et al [65] have shown that C6 glioma cells, exposed to a gradual change in the external osmolality, require a larger decrease to stimulate the

release of taurine compared to that required to stimulate an increase in Cl⁻ permeability. Similarly, Tomassen et al [29] have shown that activation of swelling-induced taurine release from cultured human intestinal cells required the medium osmolality to be lowered to 225 mosmoles/kg whereas volume-sensitive I⁻ efflux was activated at 290 mosmoles/kg. It is perhaps possible, in some instances, that volume-activated taurine release is used to combat larger perturbations to the cellular hydration state.

Other evidence in favour of separate volume-activated taurine and Cl⁻ channels includes differential regulation and inhibition of taurine and Cl⁻ fluxes both within and between cell types. For example, DIDS inhibits swelling-induced I⁻ efflux but not volume-activated taurine release from intestinal 407 cells [29]. Conversely, DIDS is more effective at inhibiting volume-activated taurine release than swelling-induced I⁻ egress from HeLa and H1H/3T3 cells [35, 61, 66]. Also, Tomassen et al [67] have shown that 1-butanol, a compound which blocks the activation of phospholipase D, markedly inhibits swelling-induced I⁻ efflux from intestine 407 cells without affecting osmosensitive taurine release. The finding that swelling-activated taurine and Cl⁻ fluxes are differentially regulated by the expression of Rho GTPases in NIH3T3 mouse fibroblasts suggests the presence of separate pathways [68]. A recent report from Lopez-Dominguez et al [69] supports this suggestion. They found that the extent of osmosensitive taurine and Cl⁻ efflux from Swiss 3T3 fibroblasts was dependent upon the manner of cell swelling. Thus a hyposmotic challenge had a larger effect on Cl⁻ release compared to that of taurine efflux whereas cell swelling induced by urea had the opposite effect. Such observations are not consistent with a single pathway.

The relationship between volume-sensitive taurine transport and the anion-exchanger AE1

A considerable number of studies advocating a role for the anion-exchanger (AE1, band 3 anion exchanger) in volume-activated taurine transport in fish erythrocytes have been published. Fugelli & Thorod [20] reported that flounder red blood corpuscles released taurine in response to a hyposmotic challenge. Soon after, it was claimed, on the basis of DIDS inhibition, that volume-activated taurine transport in fish erythrocytes was via the band 3 anion-exchanger [70, 71]. However, the finding that

sulphate efflux via the band 3 protein was not stimulated by cell swelling was taken as evidence that volume-sensitive taurine transport could not simply be attributed to an increase in anion-exchange activity [71]. In view of the finding that volume-sensitive taurine transport was non-saturable, it was suggested that a hyposmotic shock induced channel activity associated with the AE1 [72].

Other evidence cited in favour of a role for the anion-exchanger in volume-sensitive taurine transport in fish red blood cells are the findings that cell swelling increases the binding of DIDS to the band 3 protein and stimulates the phosphorylation of the exchanger [73]: these results could be explained by the fact that cell swelling induces the movement of AE1 from an intracellular pool to the plasma membrane [74]. In addition, it has been reported that osmotic swelling induces the formation of band 3 tetramers in skate erythrocytes [75, 76]. The observation that cell swelling does not increase taurine efflux from hagfish erythrocytes, a cell type lacking band 3, has also been used to support the argument that the anion-exchanger plays a pivotal role in volume-activated taurine release [77]. Even though a role for AE1 in volume-activated taurine transport in skate erythrocytes was suggested by Goldstein and colleagues, they suggested, in contrast to Kirk et al [38] that volume-sensitive taurine and Cl⁻ transport in fish erythrocytes occurred via separate pathways [78].

None of the above findings, either alone or combined, constitute direct proof that band 3 acts as a conduit for volume-activated taurine in fish red blood cells. However, an apparent major breakthrough came with the report that expression of trout AE1 (tAE1) in *Xenopus* oocytes induced a chloride conductance (as well as anion-exchange activity) and increased radiolabelled taurine transport [79]. At first sight this appears to be evidence showing that osmosensitive taurine and Cl⁻ efflux is associated with the Band 3 protein. Either AE1 is itself the site of volume-activated taurine transport or alternatively, it could be that endogenous osmosensitive flux pathways in *Xenopus* oocytes are under the control of expressed tAE1. In this connection, Koomoa et al [80] suggested that tAE1 acts as the channel: this notion is based on the finding that the endogenous volume-activated taurine transport pathway in *Xenopus* oocytes exhibited different properties from that associated with tAE1.

However, it is difficult to reconcile AE1 with volume-activated taurine transport given that taurine and Cl⁻ transport induced by the expression of tAE1, is not affected by hypotonicity. As a means of explaining this somewhat perplexing finding, it has been suggested that

tAE1 may adopt a new conformation when expressed in *Xenopus* oocytes [79, 80]. Equally baffling is the observation that taurine transport in oocytes expressing tAE1, unlike that found in trout red blood cells, is insensitive to DIDS. The association between band 3 and volume-sensitive taurine efflux appeared to be strengthened by the report that the expression of skate AE1 in *Xenopus* oocytes activated osmosensitive taurine transport [81, 82]. However, this result is controversial given that Guizouarn et al [83] could not find an increase in taurine transport in oocytes expressing skate AE1 compared with non-injected control oocytes.

There are several lines of evidence which appears to rule out an association between mammalian AE1 and volume-activated taurine transport. First, human erythrocytes, despite having an abundance of the band 3 protein, do not exhibit volume-activated taurine transport [37]. Secondly, mouse AE1 expressed in *Xenopus* oocytes failed to stimulate taurine transport [79]. Thirdly, Sanchez-Olea et al [84] found that the extent of volume-activated taurine efflux in Chinese hamster ovary cells was independent from the expression of AE1. However, there are circumstances under which mammalian AE1 appears to act as an anion (taurine) channel. Thus, a construct of the human AE1 protein lacking putative trans-membrane domains 6 and 7 is associated with an anion conductance when expressed in *Xenopus* oocytes [85]. It appears that the AE1 construct is able to induce the transport of taurine which is partly sensitive to the anion channel blocker flufenamate. Again it is difficult to discern whether the human AE1 construct is itself acting as a taurine permeable channel or is stimulating an endogenous taurine channel.

There are, however, two reports which advocate a role for the anion exchanger in mammalian cell volume regulation. Conejero [86] has suggested that AE1 may be a candidate pathway for volume-sensitive taurine transport in rat red blood cells although the results remain to be confirmed. The claim is largely based on pharmacological evidence which, as discussed above, does not constitute direct proof of a shared pathway since anion transport inhibitors are non-specific. Law [87] has proposed that inwardly directed HCO_3^- transport via $\text{Cl}^-/\text{HCO}_3^-$ exchange operating in parallel with taurine efflux via taurine/ Cl^- exchange is able to facilitate an RVD in rat cerebral cortical cells. It is hypothesized that the conversion of bicarbonate to CO_2 and water reduces the intracellular osmotic pressure and thus underpins the recovery in cell volume.

Do neutral α -amino acids share a pathway with taurine?

It is evident that a hyposmotic shock respectively reduces and increases the intracellular and extracellular concentration of non-essential α -amino acids such as L-alanine and glycine (e.g. see [14, 43, 88, 89]). This is consistent with volume-activated α -amino acid efflux. In support of this, it has also been shown that cell swelling increases the unidirectional efflux of radiolabelled glycine and L-alanine [14, 90-92]. It has been suggested, on the basis of inhibitor studies, that α -neutral amino acids utilize the volume-activated taurine efflux pathway [89, 92]. Thus volume-activated taurine and glycine efflux from CHP-100 cells is inhibited by NPPB [92] and osmosensitive taurine, glycine and L-alanine efflux from cardiac cells is sensitive to DIDS [89]. Both studies made the assumption that the shared pathway is an anion channels although the existence of separate pathways cannot be discounted. However, Manolopoulos et al [43] have shown, using electrophysiological techniques, that glycine is able to permeate a DIDS and NPPB sensitive volume-activated anion channel in endothelial cells. Nevertheless, it is clear that more work is required before firm conclusions can be drawn about the relationship between α -neutral amino acids and volume-sensitive taurine efflux.

Do anionic amino acids permeate the volume-activated taurine efflux pathway?

The interest in swelling-induced glutamate and aspartate efflux is fuelled primarily by the link between excitatory amino acids and brain pathology (e.g. [93-95]). It is well established that a hyposmotic challenge is able to increase the efflux of glutamate and aspartate from neuronal and glial cells [93]. Unmasking the identity of the osmosensitive glutamate/aspartate efflux pathway could possibly help attenuate the severity of the complications associated with brain ischemia and trauma. Osmosensitive release of anionic amino acids is not confined to those of the brain: it appears to be a widespread phenomenon given that it has also been observed in epithelial and cardiac tissue (e.g. see [89, 91]). One major question which arises is: do anionic amino acids share a pathway with taurine? As with the other questions posed in this review there is no simple answer. There is evidence both for and against the hypothesis that taurine and acidic amino acids share an osmosensitive pathway.

The notion that the volume-sensitive anionic amino and taurine efflux pathways are a single entity is closely related to the proposal that volume-activated amino acid transport is via volume-sensitive Cl⁻ channels (see above). In this regard, there are many reports of chloride channels exhibiting a significant permeability to glutamate and aspartate. For example, the relative glutamate/chloride permeability of volume-activated Cl⁻ channels in MDCK and IMCD cells is respectively 0.18 and 0.1 [48, 52]. In addition, several studies have shown that swelling-induced aspartate and glutamate efflux have a similar if not identical pharmacological profile to volume-activated taurine and Cl⁻ release: the osmosensitive efflux of anionic amino acids can, in many instances, be inhibited by anion channel blockers [92, 93]. The simplest explanation of some of the available data is that the efflux of anionic amino acids and taurine is via a common pathway which may indeed be a volume-activated Cl⁻ channel.

Even though a cell may exhibit volume-sensitive taurine, glutamate and Cl⁻ efflux, separate pathways cannot be discounted [19]. The finding that the acute application of tamoxifen to cultured rat astrocytes completely inhibits volume-activated Cl⁻ efflux without affecting osmosensitive D-aspartate release supports this view [95]. Indeed, there are several instances whereby taurine efflux can be elicited without a concomitant release of anionic amino acids. A hyposmotic shock increases taurine efflux but not that of glutamate and aspartate (or Cl⁻) from lactating rat mammary explants and the perfused *in situ* mammary gland [27, 58, 96, 97]. Furthermore, swelling induces the release of radiolabelled taurine but not glutamate from a human neuroblastoma cell line (CHP-100) [92]. The same study demonstrated, using patch-clamp analysis, that CHP-100 cells express swelling-activated Cl⁻ channels. Curiously, the channels displayed a small permeability to glutamate despite the fact that the efflux of radiolabelled glutamate could not be stimulated by cell swelling. Nevertheless, the results of Basavappa et al [92] could be taken as evidence for the presence of a volume-activated anion channel which is permeable to taurine but not glutamate. Similarly, cell swelling, induced by a hyposmotic shock, increases taurine efflux from rat pituitary cells without affecting L-glutamate release [98, 99]. Swelling-induced release of radiolabelled taurine and D-aspartate from cultured astrocytes is differentially regulated by tyrosine kinases in a manner which points to the existence of a pathway which is permeable to taurine but not anionic amino acids [100]. Thus, the tyrosine kinase inhibitors tyrphostin 23 and tyrphostin A51 partially inhibit volume-activated taurine release without affecting D-

aspartate efflux. There is also the strong possibility that there are separate volume-activated taurine and D-aspartate efflux pathways in rat hippocampal slices and chick retina. NPPB inhibits osmosensitive taurine but not D-aspartate efflux from hippocampal slices [36] and it is apparent that the activation threshold for volume-sensitive taurine release from chick retinal cells is lower than that for aspartate efflux [101]. It is also possible that taurine and anionic amino acids utilize separate volume-sensitive efflux pathways in cultured pancreatic β -cells [34]: DIDS (1 mM) inhibits volume-activated taurine efflux from pancreatic INS-1 cells by 83% whereas a similar concentration of the stilbene derivative blocks the volume-sensitive component of D-aspartate release by only 60% [34]. If the assumption is made that DIDS inhibits a pathway shared by both taurine and D-aspartate then it appears that D-aspartate is also able to traverse the plasma membrane via a second pathway which is not permeable to taurine.

Inhibition of volume-sensitive taurine efflux by DIDS and tamoxifen: evidence for more than one pathway

Many studies have shown that hyposmotically-induced taurine efflux can be inhibited by DIDS and/or tamoxifen (see e.g. [22, 38, 43, 90, 92, 101-103]). As discussed above, inhibition by either compound is commonly cited as evidence for taurine transport via volume-activated anion channels. Inhibition of volume-sensitive taurine release by DIDS is also used to support the argument that the anion-exchanger (AE1) plays a role in osmosensitive taurine transport. However, the inhibition of taurine efflux by DIDS is by no means absolute or universal suggesting that there is more than one type of volume-activated taurine efflux pathway. Similarly, there are several reports of tamoxifen-insensitive, swelling-activated taurine release which also supports the notion that there are at least two routes for taurine release.

A hyposmotic shock increases Na⁺-independent taurine efflux from mouse 1-cell conceptuses and blastocysts [104]. However, whilst DIDS inhibits volume-activated taurine release from day-1 conceptuses it has no effect on taurine efflux from blastocysts even at the high concentration of 1 mM. This suggests that day-1 conceptuses and blastocysts express different volume-sensitive taurine release pathways. Similarly, DIDS has little or no effect on osmosensitive taurine egress from

human erythroleukemia cells [103]. There are also several reports of incomplete inhibition of volume-activated taurine efflux by DIDS. The stilbene derivative inhibits swelling-induced taurine efflux from MDCK cells by approximately 50%. A large portion of the remaining flux is sensitive to quinidine [42] suggesting that there are at least two, possibly three, swelling-activated taurine efflux pathways in this cell line. Volume-activated taurine transport in estrogen receptor-negative MDA-MB-231 human breast cancer cells is relatively insensitive to DIDS: approximately 50% of the volume-sensitive flux remains in the presence of 1 mM DIDS [33]. In contrast, DIDS almost abolishes volume-activated taurine efflux from estrogen receptor-positive MCF-7 human breast cancer cells [33]. It would appear, therefore, that MDA-MB-231 cells possess more than one swelling-induced taurine efflux pathway.

Tamoxifen has been shown to be a high-affinity inhibitor of swelling-induced taurine efflux from a variety of cells. For example, a concentration of 10 μ M is able to almost fully block swelling-induced taurine transport in bovine articular chondrocytes [22] and HeLa cells [40]. There are, however, several reports of tamoxifen-insensitive, volume-activated taurine efflux. Osmosensitive taurine efflux from human placental tissue explants is inhibited by DIDS, NPPB and niflumate but not by tamoxifen [91]. Similarly, volume-activated taurine release from rat supraoptic glial cells, pituicytes and A6 kidney cells is blocked by NPPB and DIDS but is insensitive to tamoxifen [25, 99, 105].

A recent report by Ullrich et al [106] provides strong evidence for the existence of two osmosensitive taurine efflux pathways in a polarized epithelial cell line. They studied the effect of cell swelling on the simultaneous release of taurine across the apical and basolateral membranes of Caco-2 cells and found that tamoxifen inhibited volume-activated taurine efflux across the apical membrane but had no effect on taurine release across the basolateral aspect.

Does cell swelling induce taurine and K⁺ transport via a single pathway?

The arguments for and against volume-activated taurine and K⁺ transport sharing a pathway are similar to those used to describe the relationship between taurine and Cl⁻ transport. Sanchez Olea et al [88] were the first to suggest that volume-activated taurine and K⁺ efflux share a common pathway. This suggestion was based on

the finding that quinidine inhibited swelling-induced taurine and K⁺ release from MDCK cells. Similarly Hall et al [22] found that volume-activated taurine and K⁺ efflux from HeLa had similar pharmacological profiles and time-courses and concluded that they utilized a single pathway which had the characteristics of an anion channel. However, the results of Hall et al [40] have to be squared against those of Stutzin et al [61, 62] which indicate that volume-activated taurine transport in HeLa cells does not utilize anion channels. It has also been proposed, again mostly on the basis of pharmacological inhibition, that a hyposmotic challenge induces the transport of taurine and K⁺(Rb⁺) via a shared pathway in trout erythrocytes [107]. Cell-swelling stimulates taurine release and activates at least two K⁺(Rb⁺) efflux pathways, one of which is blocked by barium, in rat hepatoma HTC cells [108]. The barium-insensitive moiety of volume-sensitive K⁺(Rb⁺) efflux shares several characteristics with the taurine efflux component: both have similar time-courses and pharmacological profiles which has led to the suggestion that they share a common pathway [108]. It has also been claimed that taurine and K⁺ efflux from human erythroleukemia cells under hyposmotic conditions share a pathway for transport. Again this conclusion is based on pharmacological evidence [103]. The finding that the expression of Rho in NIH3T3 fibroblasts has a similar effect on volume-activated taurine and K⁺ efflux does at first sight suggest the presence of a common pathway [68]. However, in none of these studies can the existence of separate pathways for taurine and K⁺ be ruled out.

Volume-activated taurine and K⁺(Rb⁺) efflux from lactating rat mammary tissue explants have similar time courses [41]. Exposing mammary tissue to a second but similar osmotic shock increases both taurine and K⁺(Rb⁺) efflux but to a lesser extent than the first. It is notable, however, that taurine efflux is reduced more than K⁺(Rb⁺) release which is not consistent with the concept of a shared pathway [41].

Other organic osmolytes may share a pathway with taurine

Cell swelling also increases the efflux of inositol, betaine, choline, uridine and glucose from a variety of cells [21, 22, 38, 40, 109-111]. Again it has been proposed that most of these compounds share a pathway with taurine. This is based on the finding that the volume-activated release of each of these osmolytes has a similar time course and almost identical pharmacological profile

Table 1. Does a single pathway account for volume-activated taurine and Cl⁻ transport?

Evidence for:	Refs.
1. Swelling-induced taurine and Cl ⁻ transport have a similar pharmacological profile (i.e. inhibition by anion channel inhibitors such as DIDS, NPPB and tamoxifen)	21, 28, 40, 48, 56, 64
2. Hypotonically-activated taurine and Cl ⁻ fluxes/currents are regulated in a similar fashion	54, 119
3. Volume-activated Cl ⁻ channels are permeable to taurine (measured electrophysiologically)	21, 48, 53-56
Evidence against:	
1. Volume activated taurine and Cl ⁻ (I ⁻) efflux have different time courses (especially with respect to inactivation)	33, 60-63
2. Some of the inhibitors used to justify a role for Cl ⁻ channels are notoriously non-specific (i.e. they also block anion-exchange and cation channels)	49
3. Osmosensitive taurine and Cl ⁻ transport have different osmotic thresholds	29, 65
4. Volume-sensitive taurine and Cl ⁻ efflux can be differentially regulated/inhibited	26, 29, 35, 61, 66-69
5. Volume-sensitive taurine efflux can be activated without a concomitant increase in Cl ⁻ efflux	27, 35, 58, 60

Table 2. Is volume-activated taurine release mediated by the band 3 protein (AE1)?

Evidence for:	Refs.
1. Volume -activated taurine release is inhibited by anion-exchange inhibitors (i.e. DIDS, niflumate, NPPB)	70-72
2. Cell swelling increases the binding of DIDS to the band 3 protein and induces the formation of band 3 oligomers	73, 75, 76
3. Expression of trout band 3 in <i>Xenopus</i> oocytes induces taurine transport	79, 80, 83
Evidence against:	
1. The inhibitors used to justify a role for band 3 are non-specific	49
2. Taurine transport induced by the expression of trout band 3 in <i>Xenopus</i> oocytes is not increased by cell swelling or inhibited by DIDS	79, 83
3. Human red blood cells, despite expressing band 3, do not exhibit volume-activated taurine transport	37

Table 3. Do anionic amino acids utilize the volume-activated taurine efflux pathway?

Evidence for:	Refs.
1. Swelling-induced taurine and anionic amino acid efflux have a similar pharmacological profile (i.e. inhibition by anion transport blockers)	89, 91-93
2. Electrophysiological studies have shown that both taurine and L-glutamate are permeable to volume-sensitive Cl ⁻ channels	48, 52
Evidence against:	
1. Cell swelling can increase taurine efflux without increasing L-glutamate/D-aspartate release	27, 58, 92, 96, 97
2. Swelling-induced taurine and anionic amino acid release can be differentially regulated	100
3. Differential inhibition of volume-sensitive taurine and D-aspartate efflux	36, 34, 95
4. Volume-activated taurine and D-aspartate efflux can have different osmotic activation thresholds	101

to that of taurine efflux. There is also evidence to support the suggestion that the volume-activated efflux of inositol is via a channel which in turn could be a volume-sensitive anion channel [21]. However, the possibility that compounds such as inositol, betaine, uridine and glucose are not obliged to utilize a taurine efflux pathway (chloride channel) remains open.

A hyposmotic challenge increases the efflux of both taurine and creatine from rat cortical astrocytes [30]. However, the time courses of taurine and creatine efflux, in response to cell swelling, markedly differed as did the effect of anion transport inhibitors. Thus, NPPB and DIDS had less of an inhibitory effect on creatine release compared with their effect on taurine efflux [30].

Fig 1. Schematic diagram showing different permutations of volume-activated taurine efflux pathways: a) taurine transport via a volume-activated anion channel which can be blocked by anion transport inhibitors such as DIDS, NPPB, tamoxifen and niflumate. Anionic amino acids can also permeate this pathway. There is the possibility that neutral amino acids and other osmolytes such as inositol, uridine and glucose may use this pathway. The most convincing evidence for this pathway comes from electrophysiological studies. b) independent osmosensitive taurine and chloride channels both of which can be inhibited by anion transport blockers. The existence of separate channels stems from the finding that cell swelling is able to increase taurine efflux without an increase in chloride permeability. However, both of these pathways may exist in a single membrane - the existence of separate pathways can also be inferred from the time-courses of taurine and Cl⁻(I⁻) transport. c) tamoxifen-sensitive and -insensitive volume-activated taurine efflux pathways. Either or both of these channels may have identity with volume-sensitive chloride channels. d) Separate osmosensitive taurine and anionic amino acid channels. There are instances whereby cell swelling can increase taurine transport without a concomitant increase in glutamate/aspartate efflux. Also, there is evidence for the differential inhibition of glutamate/aspartate and taurine efflux from some cells. In particular, DIDS can reduce taurine efflux without readily affecting anionic amino acid release.

It appears therefore that osmosensitive taurine and creatine do not share a pathway in rat cortical cells.

Concluding remarks

It is apparent from this review that the discussion about the identity of the swelling-induced taurine efflux pathway and its relationship with other volume-sensitive solute efflux pathways is far from settled. (see tables 1-3). The field has become complicated and controversial since it was first proposed that volume-activated taurine efflux is via a swelling-induced Cl⁻ channel and/or the band 3 anion-exchanger. It is nigh impossible to form a unifying hypothesis in face of the available data. On the

contrary, the evidence supports the notion that there may be more than one, possibly multiple, volume-activated taurine efflux pathways (see figure 1):

It appears that the suggestion that swelling-induced taurine efflux is via volume-activated anion channels does not hold for all cell types. Whilst there is *prima facie* evidence for a shared pathway there is also an abundance of evidence suggesting otherwise. In this regard, the practice of using taurine as a Cl⁻ congener to study volume-sensitive anion channels should be discontinued unless it has been established beyond doubt that taurine and Cl⁻ share a pathway in the cells under investigation. In addition, the demonstration that a cell possesses hypotonically-induced acidic amino acid efflux should not by itself be used as evidence for the presence of volume-

sensitive organic anion channels (e.g. see [112]). Furthermore, electrophysiological studies of volume-activated anion channels should also be routinely supported by tracer efflux experiments. Even though patch-clamp studies may show that amino acids such as taurine and L-glutamate permeate volume-activated Cl⁻ channels, the significance of such an observation needs to be assessed under more physiological conditions.

The role of the anion-exchanger (AE1; band 3) in volume-activated taurine transport is still not clear. The argument in favour of the AE1 acting as a conduit for swelling-induced taurine transport is strong when applied to fish cells. However, it is apparent that the AE1 does not facilitate volume-sensitive taurine transport in mammalian cells with the possible exception of rat erythrocytes [86]. More work is required to establish whether AE1 is itself the taurine channel in fish red cells or alternatively if AE1 is acting as a regulator, possibly via the cytoskeleton, of volume-activated taurine release. Finding precise answers to the major questions raised in this review will be greatly facilitated by the cloning and

expression of volume-activated transport systems. In this connection, the molecular identity of the most studied category of volume-sensitive pathway, namely volume-activated Cl⁻ channels, is not known. Several proteins including P-glycoprotein, CIC-2, CIC-3 and pI_{Cl⁻} have been proposed but none of the candidates has gained widespread acceptance [113, 114]. Phospholemman, a protein which has a single membrane-spanning domain, was suggested to be a volume-activated taurine (anion) channel [115]. However, it is not yet clear if phospholemman is itself a taurine channel or a modulator of volume-activated taurine transport [116, 117]. The structure of the protein would, at first sight, appear to favour the latter suggestion. However, a recent report suggests that phospholemman tetramers have the potential to form a hydrophilic pore which could accommodate taurine [118]. It can be predicted, on the basis of evidence gathered from functional investigations, that molecular studies could eventually reveal the existence of more than one system for osmosensitive taurine transport.

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