Volume-regulatory taurine release from a human lung cancer cell line

Evidence for amino acid transport via a volume-activated chloride channel

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Exposure of a human lung epithelial cancer cell-line to hypo-osmotic media led to a marked increase in the rate of efflux from the cells of taurine, a non-essential sulfonic amino acid. The osmotically-activated taurine efflux was inhibited by a range of known Cl^- channel blockers, the most potent of which were NPPB and 1,9-dideoxyforskolin. These reagents were similarly effective at inhibiting the osmotically-activated efflux of I^-, a known substrate of volume-activated Cl^- channels. The results are consistent with the hypothesis that volume-regulatory taurine release from these cells is mediated by a volume-activated Cl^- channel.

Taurine; Volume regulation; RVD; Volume-activated chloride channel; Membrane transport

1. INTRODUCTION

Most cell-types have the ability to regulate their volume following cell swelling. An increase in cell volume triggers the activation of membrane transport pathways which mediate the net efflux of cytoplasmic solutes; this results in the cell undergoing a regulatory volume decrease (RVD). Most studies of RVD mechanisms have focused on the role of K^+ and Cl^-, which escape from the cell via separate volume-activated channels and/or a volume-activated KCl cotransporter [1,2]. However, many cells, when swollen, also release small organic solutes and this process often makes a substantial contribution to the total RVD. In vertebrate cells the primary organic 'osmolyte' is usually taurine, a sulfonic amino acid which may be present in the cytoplasm at concentrations of up to 40 mM [3]. Volume-activated taurine release has been demonstrated from many vertebrate cells and tissues including fish erythrocytes, heart, brain and hepatocytes [4–12], bird erythrocytes [13], Ehrlich ascites tumour cells [14–16], MDCK cells [17,18], rabbit lymphocytes [19], and a variety of mammalian brain cell-types [20–24]. These studies have revealed a number of common characteristics for the transport mechanisms which mediate volume-activated taurine release from different cells: they are, in general, Na^+-independent, non-saturable and not prone to

'trans-stimulation' [6,15,18,22,25]. In many cases volume-activated taurine efflux is accompanied by the release of other amino acids including glutamate, aspartate, β-alanine and glycine [15,17,18,21], suggesting that the pathways are not highly selective. However there is evidence that they have a relatively low permeability to cationic amino acids [6,18].

Amino acids are not the only organic osmolytes used by mammalian cells. On prolonged exposure to hyperosmotic media a number of mammalian renal and brain cell-types accumulate high concentrations of polyols, predominantly sorbitol and inositol [26–29]. When returned to iso-osmotic solution the cells swell and respond by releasing these compounds, thereby undergoing RVD [26,29–34]. The volume-activated sorbitol and inositol transport mechanisms are Na^+-independent and non-saturable [30,34]. They accommodate a number of different polyols [30] and show pharmacological similarities to the pathways which mediate volume-regulatory amino acid release from other cell-types [34].

In a recent study of fish (flounder) erythrocytes we obtained data consistent with the hypothesis that the volume-activated taurine transport pathway in these cells is permeable to the monosaccharide glucose and to the nucleoside uridine [25]. The very broad substrate specificity of this pathway, its Na^+-independence and its failure to saturate or to show trans-stimulation suggest that it behaves less like a conventional amino acid transporter than a pore or channel. The pharmacological properties of this pathway are very similar to those of 'Cl^- channels' in other cells [25] and it has recently proposed that the volume-regulatory efflux of amino acids and of other organic osmolytes from a number of different cell-types is via channels of this sort [18,34,35].

In the work reported here we have demonstrated volume-activated taurine release from a human epithelial lung cancer cell line, and have made a direct comparison of the pharmacological properties of the taurine pathway with those of a pathway which mediates the swelling-activated release of I^-, a known substrate of volume-activated Cl^- channels. The results further support the hypothesis that volume-activated Cl^- channels mediate the transport of small organic solutes and that this represents an important aspect of their physiological function.

2. MATERIALS AND METHODS

2.1. Materials

Cell culture reagents were from ICN Biomedicals. [^{14}C]Taurine was from DuPont New England Nuclear. 1,9-Dideoxyforskolin, glibenclamide, verapamil, quinine and 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) were from Sigma and 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) was a generous gift from Prof. R. Greger (Physiologisches Institut der Albert-Ludwigs-Universitat, Freiburg, Germany). All inhibitors were made up as stock solutions (60 mM) in dimethyl sulfoxide.

2.2. Cell culture

The human lung epithelial cancer cell line, S1 [36], was maintained as a monolayer culture in HAMS F12 medium supplemented with 10% v/v fetal calf serum and 2 mM glutamine. Cells were grown in 5% CO_2 under 100% humidity at 37°C and maintained in exponential growth phase by passaging twice weekly. Exponentially growing cells were trypsinised, centrifuged and resuspended in fresh growth medium. Cell suspension (2 ml) was aliquoted into 35 mm diameter tissue culture dishes and allowed to grow to 60–90% confluence.

2.3. Solutions

Efflux of taurine and I^- from S1 cells was measured under both iso-osmotic and hypo-osmotic conditions. In the initial taurine efflux experiments the iso-osmotic solution contained 150 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM HEPES and 5 mM glucose and was pH-adjusted to 7.4. In the hypo-osmotic solution the NaCl concentration was reduced to 95 mM, while the other components remained unchanged. Later taurine efflux experiments were carried out in solutions designed to minimise the ability of the cells to undergo RVD in the hypo-osmotic media. In these experiments the iso-osmotic solution contained 55 mM NaCl, 85 mM KCl, 25 mM taurine, 1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM HEPES and 5 mM glucose and was pH-adjusted to 7.4. In the hypo-osmotic solution NaCl was omitted, while the other components remained unchanged.

I^- efflux was monitored using an I^--sensitive electrode (HNU systems, USA); these experiments were therefore carried out using halide-free solutions in order to minimise interference with the I^- measurements. The iso-osmotic solution contained 150 mM $NaNO_3$, 5 mM KNO_3, 1.3 mM $Ca(NO_3)_2$, 0.5 mM $Mg(NO_3)_2$, 10 mM HEPES and 5 mM glucose and was pH-adjusted to 7.4. In the hypo-osmotic solution the $NaNO_3$ concentration was reduced to 95 mM, while the other components remained unchanged.

The osmolality of the solutions was measured using a freezing-point osmometer (Roebling, Germany). The osmolality of the iso-osmotic solutions was, in all cases, within the range 295–310 mosm · kg^{-1}, and that of the hypo-osmotic solutions, in all cases, within the range 200–210 mosm · kg^{-1}.

2.4. Taurine efflux

Cells grown to 60–90% confluence in 35 mm diameter dishes were loaded with [^{14}C]taurine by incubation for 1–3 h at 37°C in standard growth medium (1 ml) containing [^{14}C]taurine at an activity of 0.1 μCi/ml. [^{14}C]Taurine efflux was measured at room temperature (~22°C). Immediately before beginning each efflux experiment the loading solution was removed and the cells were washed (6–10 times) by the repeated addition then removal of 1 ml of iso-osmotic saline, over a 4–8 min period. Wash solutions were discarded. On removal of the final wash a further 1 ml of iso-osmotic solution was added to the dish. After either 1 or 2 min the solution was removed and replaced immediately with another 1 ml aliquot. The solution removed from the culture dish was transferred directly to a scintillation vial containing 2 ml of scintillant. This procedure was repeated throughout the duration of the experiment, with the iso-osmotic solution being replaced with solutions of different composition as required. On completion of the time-course, 1 ml of 0.5 M NaOH was added to each dish to lyse the cells and thereby release the remaining intracellular taurine. After 5 min the lysate was transferred to a scintillation vial and the dish rinsed (×5) with 1 ml aliquots of H_2O which were also transferred to vials for β-scintillation counting.

Taurine efflux time-courses were analysed by plotting $\ln(Taurine_i(t)/Taurine_i(t = 0))$ versus time, t. $Taurine_i(t = 0)$ denotes the total amount of [^{14}C]taurine present inside the cells at the beginning of the efflux time-course and was obtained by summing the amount of radioactivity (dpm) in all of the samples taken throughout the time-course together with that remaining in the NaOH cell extracts and H_2O rinses. $Taurine_i(t)$ denotes the amount of [^{14}C]taurine remaining inside the cells at time t and was obtained by subtracting the sum of the radioactivity in the samples taken up to time t from the total amount originally present inside the cells. The negative slope of the graph of $\ln(Taurine_i(t)/Taurine_i(t = 0))$ versus t provides a measure of the unidirectional rate constant for taurine efflux.

2.5. Iodide efflux

Cells grown to 60–90% confluence in 35 mm diameter dishes were loaded with I^- by incubation for 1–2 h at 37°C in a solution (1 ml) comprised of 140 mM NaI, 5 mM KI, 1.3 mM $Ca(NO_3)_2$, 0.5 mM $Mg(NO_3)_2$, 20 mM HEPES and 5 mM glucose and supplemented with 20% v/v fetal calf serum. I^- efflux experiment were carried out at room temperature using a protocol very similar to that used in the [^{14}C]taurine experiments. The loading solution was removed and the cells washed (×6) in iso-osmotic (halide-free) solution before commencing the efflux time-course with the addition of a further 1 ml aliquot of solution. At 1 min intervals the solution was removed and immediately replaced with another 1 ml aliquot, with the composition of the added solution being altered as required. The solutions removed from the culture dish were, for convenience, transferred to 24-well plates and the I^- concentration in each subsequently measured using an I^--sensitive electrode.

At the conclusion of the I^- efflux time-courses a 1 ml aliquot of hypo-osmotic halide-free saline was added to the cells and left for at least 1 h before being transferred to the 24 well plate and replaced with another 1 ml aliquot of hypo-osmotic saline. This was repeated twice then the dishes rinsed twice more with 1 ml aliquots of hypo-osmotic saline. The I^- present in the three final (>1 h) incubation samples, together with that in the final rinse solutions provided a measure of the total free (diffusible) I^- remaining in the cells at the conclusion of the time-course experiment.

I^- efflux time-courses were analysed by plotting $\ln(I_i^-(t)/I_i^-(t = 0))$ versus time, t. $I_i^-(t = 0)$ denotes the total diffusible I^- present inside the cells at the beginning of the efflux time-course and was obtained by summing the concentration of I^- in each of the 1 ml samples taken throughout the time-course, together with that in the final (>1 h) incubation samples and rinse solutions. $I_i^-(t)$ denotes the amount of I^- remaining inside the cells at time t and was obtained by subtracting the sum of the I^- concentrations in the samples taken up to time t from the total amount of I^- originally present inside the cells. The negative slope of the graph of $\ln(I_i^-(t)/I_i^-(t = 0))$ versus t provides a measure of the unidirectional rate constant for I^- efflux.

3. RESULTS

Fig. 1 shows the effect of extracellular osmolality on the time-course for efflux of [^{14}C]taurine from S1 cells. The cells were bathed initially in iso-osmotic solution which, at the point indicated, was replaced by hypo-osmotic solution containing 95 mM NaCl. Following the exposure of cells to this solution there was a short (2–3 min) lag, followed by a marked increase in the taurine efflux rate. The osmotic activation of taurine efflux was readily reversible; on replacement of the hypo-osmotic solution with the original iso-osmotic medium the efflux reverted to its original value. On return of the cells to hypo-osmotic media, taurine efflux again increased after a short lag; however, the rate of influx was less than that following the initial hypo-osmotic exposure. When the cells were returned to the iso-osmotic solution the flux again reverted to its original level and on subsequent exposure of the cells to hypo-osmotic solution the rate of taurine efflux increased only slightly.

Fig. 2 illustrates the effect of the composition of the hypo-osmotic solution on the rate of decline of the taurine efflux rate constant following hypotonic exposure. On prolonged incubation of the cells in a hypo-osmotic solution containing Na$^+$ as the primary cation the taurine efflux rate gradually decreased, returning to its original value, usually within 40 to 80 min (Fig. 2, closed symbols). A different pattern of behaviour was seen for cells exposed to a hypo-osmotic solution containing KCl (85 mM) + taurine (25 mM) in place of
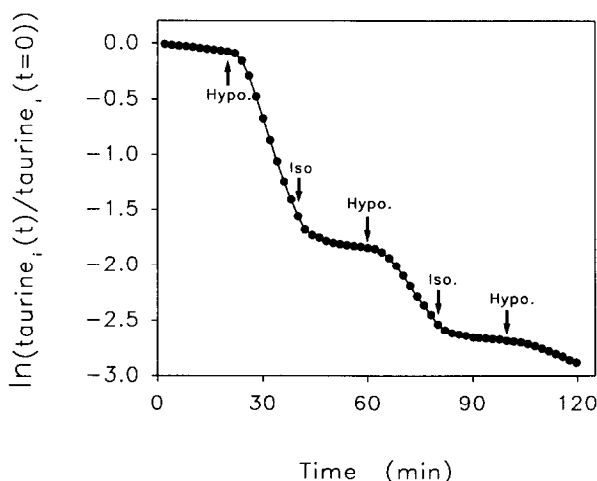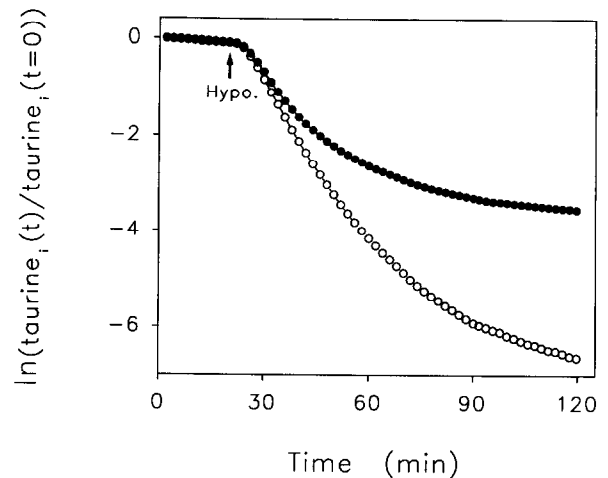
Fig. 2. Effect of the composition of the extracellular medium on the osmotically-activated efflux of taurine from S1 cells. The cells were bathed initially in iso-osmotic medium which, at the point indicated ($t = 5$ min) was replaced with hypo-osmotic medium. In the time-course represented by the closed circles the iso- and hypo-osmotic media were as for Fig. 1. In the time-courses represented by the open circles the composition of the media was altered to minimise the ability of the cells to undergo RVD following hypotonic swelling; the iso-osmotic solution contained 55 mM NaCl, 85 mM KCl, 25 mM taurine, 1.3 mM CaCl$_2$, 0.5 mM MgCl$_2$, 20 mM HEPES and 5 mM glucose (pH 7.4), while in the hypo-osmotic solution NaCl was omitted. The data shown are from a single experiment and are representative of those obtained in three similar experiments with different cell preparations.

Fig. 1. Effect of medium osmolality on the efflux of taurine from S1 cells. The graph shows the ln of the fraction of original intracellular taurine remaining as a function of time; the negative slope of the line is therefore equivalent to the unidirectional efflux rate constant. The bathing medium was alternated between iso-osmotic solution (150 mM NaCl, 5 mM KCl, 1.3 mM CaCl$_2$, 0.5 mM MgCl$_2$, 20 mM HEPES, 5 mM glucose; pH 7.4) and hypo-osmotic solution (in which NaCl was reduced to 95 mM) as indicated. The data are from a single experiment and are representative of those obtained in five similar experiments with different cell preparations.

NaCl (Fig. 2, open circles). For cells in this solution the (normally outward) electrochemical gradients for K$^+$ and taurine were reduced or even reversed and the cells were therefore prevented from undergoing RVD (e.g. [10]). Under these conditions the *initial* increase in the rate of taurine efflux following the reduction in osmolality was approximately the same as that seen in the low-K$^+$ medium; however the enhanced efflux rate persisted for very much longer and had not returned to basal levels even after 2 h.

All subsequent experiments were carried out using the high-K$^+$/taurine solution in order to prolong the osmotically-activated response. In repeated experiments, carried out under these conditions the taurine efflux rate constant increased, on average, from 0.37 ± 0.06 h^{-1} ($\pm$ S.E.M., $n = 15$) in isotonic media (295–310 mosm · kg^{-1}) to 4.0 ± 0.9 h^{-1} ($n = 15$) in hypotonic media (200–210 mosm · kg^{-1}), an increase of more than 10-fold.

A variety of known Cl$^-$ channel blockers were tested for their effect on osmotically-activated taurine release (Table I). 1,9-Dideoxyforskolin, recently identified as an effective inhibitor of the osmotically-activated Cl$^-$ channel associated with the human *mdr1* gene product, P-glycoprotein [37,38], was a potent inhibitor of osmotically-activated taurine efflux. Other P-glycoprotein inhibitors, verapamil and quinine, were somewhat less effective, as was glibenclamide, a recently identified in-
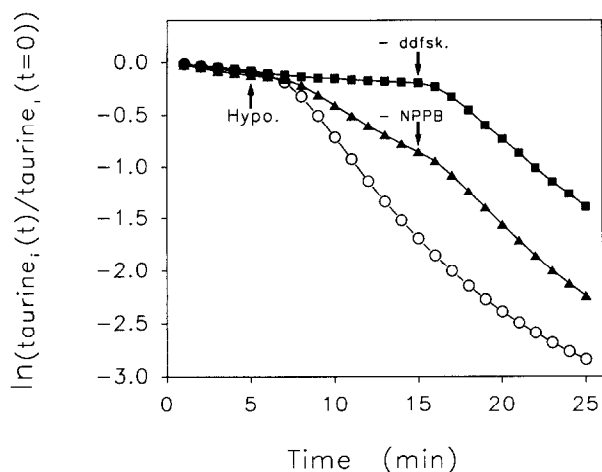
Fig. 3. Reversible inhibition of osmotically-activated taurine efflux by 1,9-dideoxyforskolin and NPPB. The cells were bathed initially in iso-osmotic (high K^+/taurine) medium which, at the point indicated ($t = 5$ min), was replaced with hypo-osmotic solution containing either 0.1 mM 1,9-dideoxyforskolin (■), 0.1 mM NPPB (▲) or no inhibitor (○). At $t = 15$ min the 1,9-dideoxyforskolin and NPPB solutions were replaced with inhibitor-free (hypo-osmotic) medium.

hibitor of the cystic fibrosis transmembrane conductance regulator, CFTR (not shown) [39]. The more widely recognised chloride channel blockers NPPB and DIDS [40] also blocked osmotically-activated taurine release, though with lesser potency than 1,9-dideoxyforskolin. The inhibitory effects of all of the blockers tested were fully reversible. Fig. 3 shows representative time-course experiments, illustrating the inhibitory effect and reversibility of the two most potent inhibitors identified, 1,9-dideoxyforskolin and NPPB, on osmoti-
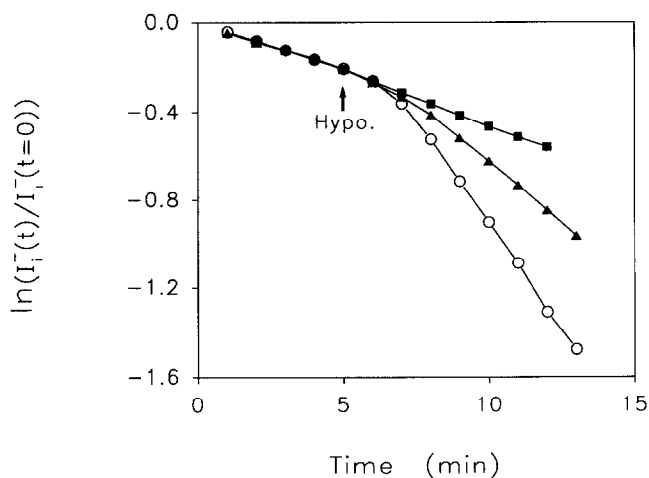

Fig. 4. Osmotically-activated I^- efflux from S1 cells. The graph shows the ln of the fraction of original intracellular I^- remaining as a function of time; the negative slope of the line is therefore equivalent to the unidirectional I^- efflux rate constant. The cells were bathed initially in iso-osmotic medium (150 mM $NaNO_3$, 5 mM KNO_3, 1.3 mM $Ca(NO_3)_2$, 0.5 mM $Mg(NO_3)_2$, 20 mM HEPES, 5 mM glucose; pH 7.4) which, at the point indicated ($t = 5$ min), was replaced with hypo-osmotic solution (in which $NaNO_3$ was reduced to 95 mM) containing either 0.1 mM 1,9-dideoxyforskolin (■), 0.1 mM NPPB (▲) or no inhibitor (○).

cally-activated taurine release from cells in a high K^+/taurine medium.

The pathway mediating osmotically-activated taurine release from S1 cells showed pharmacological characteristics very similar to those of volume-activated Cl^- channels in other cell-types. In order to confirm the presence of such channels in these cells, and to make a quantitative comparison between the pharmacological properties of these channels and those of the taurine release pathway, we measured the efflux of I^- under iso-osmotic and hypo-osmotic conditions. I^- is a useful congener for Cl^- in the study of volume-activated Cl^- channels as such channels show a higher permeability to I^- than to Cl^- itself, whereas I^- is a poor substrate for anion exchange mechanisms and cation–anion cotransporters [41].

Fig. 4 shows representative time-courses for the efflux of I^- from S1 cells. On reduction of the osmolality of the extracellular solution there was, after a short lag, a marked increase in the rate of I^- efflux. On average the I^- efflux rate constant increased from 3.1 ± 0.5 h^{-1} ($n = 14$) under isotonic conditions to 10.1 ± 1.2 h^{-1} ($n = 14$) in hypotonic media. Osmotically-activated I^- release was inhibited by NPPB and by 1,9-dideoxyforskolin: 0.1 mM NPPB reduced osmotically-activated I^- efflux to $52 \pm 9\%$ its control value ($n = 8$); 0.1 mM 1,9-dideoxyforskolin was more effective, reducing the osmotically-activated I^- efflux to $11 \pm 4\%$ its control value ($n = 10$). Neither inhibitor had any significant effect on I^- efflux from cells under iso-osmotic conditions (not shown). The percentage inhibition of osmotically-activated I^- efflux by 0.1 mM NPPB and by 0.1 mM 1,9-dideoxyforskolin was not significantly different from that of osmotically-activated taurine efflux ($P > 0.05$, paired t-test).

Fig. 5 shows dose–response curves for the effect of 1,9-dideoxyforskolin on the osmotically-activated components of taurine and I^- efflux. The two curves are superimposable, with an IC_{50} value of 63 ± 5 μM for taurine and 59 ± 6 μM for I^-. There was no significant difference ($P > 0.05$, paired t-test) between the mean

Table I

Effect of Cl^- channel blockers (0.1 mM) on osmotically-activated taurine efflux from S1 cells. The osmotically-activated efflux component was calculated by subtracting the rate constant for taurine efflux in iso-osmotic medium from the maximum rate constant attained following reduction of the osmolality and is expressed as a percentage of that measured in the absence of inhibitors. The data are averaged from 4–10 experiments similar to those illustrated in Fig. 3.

Inhibitor	Volume-activated taurine efflux (% of control)
Quinine	80 ± 7 ($n = 4$)
Verapamil	76 ± 6 ($n = 5$)
DIDS	61 ± 5 ($n = 5$)
NPPB	39 ± 7 ($n = 8$)
1,9-Dideoxyforskolin	19 ± 4 ($n = 10$)

IC_{50} values for the two substrates, nor between the percentage inhibition of the volume-activated fluxes of the two substrates at any of the inhibitor concentrations tested. The pharmacological data are therefore consistent with the hypothesis that osmotically-activated efflux of taurine and I^- from these cells was via a common pathway.

4. DISCUSSION

Exposure of mammalian cells to hypo-osmotic media causes them to swell. Osmotic swelling of human lung epithelial cancer (S1) cells was shown here to result in the activation of a pathway which mediated the efflux of the non-essential sulfonic amino acid, taurine. In this respect S1 cells resemble a wide variety of other mammalian cell-types including epithelial [17,18], brain [20,22,24] and blood [19] cells, as well as numerous lower vertebrate and marine invertebrate cells (reviewed by Chamberlin and Strange [42]). In S1 cells swollen in a low K^+ saline the volume-activated efflux component declined steadily with time so that within 40–80 min the flux in the hypo-osmotic solution had reverted to the basal level measured under iso-osmotic conditions (Fig. 2). By contrast, in cells swollen in a medium designed to 'clamp' the volume (containing KCl + taurine in place of NaCl) the high rate of taurine efflux attained 2–3 min after the initial exposure persisted for much longer. This suggests that the time-dependent inactivation of the pathway seen in the low K^+ medium
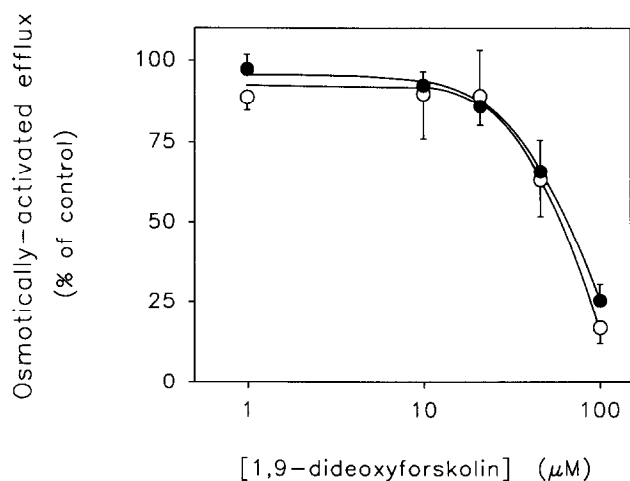

Fig. 5. Dose–response curves for the effect of 1,9-dideoxyforskolin on osmotically-activated taurine (●) and I^- (○) efflux from S1 cells. For both substrates the osmotically-activated efflux component was calculated by subtracting the rate constant for efflux in iso-osmotic medium from the maximum rate constant following reduction of the osmolality and is expressed as a percentage of that measured in the absence of inhibitors. The data are averaged from 7 different experiments. The curves were drawn using an equation of the form (% control) = $(A–D)/(1 + ([inhibitor]/C)^B) + D$, fitted to the averaged data by non-linear least squares regression. The IC_{50} values ($\pm$ S.E.M.) estimated from the averaged data were 63 ± 5 and $59 \pm 6 \,\mu M$ for taurine and I^-, respectively.

(Figs. 1 and 2) was due largely to the cells undergoing RVD during the period of hypo-osmotic exposure.

Volume-activated amino acid transport has been shown in a variety of different vertebrate cell-types to be mediated by a Na^+-independent, non-saturable 'leak' pathway, with the properties of a pore or channel rather than those of a conventional carrier or transporter [6,15,18,22,25]. Although we cannot rule out the possibility that the inhibitors used in this study interfered with the regulation of volume-activated transport, rather than with the transport pathways themselves, the simplest explanation consistent with the data obtained here is that osmotically-activated taurine release from S1 cells was via the same pathway that mediated osmotically-activated I^- (Cl^-) efflux. This pathway is most likely a volume-activated Cl^- channel. The pharmacological properties of this pathway are very similar to those of volume-activated Cl^- channels elsewhere. In particular, they show a close resemblance to those of the channel associated with expression of the human mdr1 gene product, P-glycoprotein, a 170 kDa membrane protein which, when expressed in mdr1-transfected cell lines, leads to an increased level of volume-activated Cl^- channel activity [37,43][1]. Whether P-glycoprotein itself actually functions as a Cl^- channel remains to be proven in reconstitution experiments; however, whatever the molecular identity of the channel associated with P-glycoprotein expression, it is an obvious candidate for the volume-activated taurine pathway.

The hypothesis that volume-activated Cl^- channels mediate the transport of small organic solutes such as amino acids has obvious implications for cell volume-regulation; it provides a possible explanation for the volume-regulatory release of a range of structurally unrelated organic osmolytes from different cell-types. It might also suggest a possible role for such channels in the process of nutrient absorption [35]. Amino acids, sugars, nucleosides and many other small organic substrates are transported across the apical membranes of absorptive epithelia via Na^+-dependent transporters. The coupled influx of Na^+ and organic solutes causes cell swelling which has been shown in a number of different cell-types to stimulate opening of volume-activated Cl^- channels [44–47]. Such channels, if located in the apical membrane (as in colonic T_{84} cells [48]) might, in addition to facilitating the volume-regulatory efflux of Cl^- and organic osmolytes, provide a high-capacity (albeit non-concentrative) route for the uptake of nutri-

[1]In the study which initially revealed the association between P-glycoprotein and a volume-activated Cl^- channel, S1 cells were found to have little or no volume-activated Cl^- channel activity [37]. This was clearly not the case for the cells used in the present work (Fig. 4). These were from the same original source [36] as those used in the earlier study [37], which might suggest that the functional expression of the channel may be dependent upon sub-culturing conditions.

ents when they are present in the lumen at sufficiently high concentrations to saturate the Na^+- dependent systems. Alternatively, or perhaps additionally, such channels in the basolateral membrane (as in rat colon [49] or in MDCK cells [50]) might provide a pathway for the passage of absorbed nutrients into the blood.

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