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Anion-selectivity of the Swelling-activated Osmolyte Channel in Eel Erythrocytes

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Abstract. Osmotic swelling of fish erythrocytes activates a broad-specificity permeation pathway that mediates the volume-regulatory efflux of taurine and other intracellular osmolytes. This pathway is blocked by inhibitors of the erythrocyte band 3 anion exchanger, raising the possibility that band 3 is involved in the volume-regulatory response. In this study of eel erythrocytes, a quantitative comparison of the pharmacology of swelling-activated taurine transport with that of band 3-mediated SO_4^{2-} transport showed there to be significant differences between them. *N*-ethylmaleimide and quinine were effective inhibitors of swelling-activated taurine transport but caused little, if any, inhibition of band 3. Conversely, DIDS was a more potent inhibitor of band 3-mediated SO_4^{2-} flux than of swelling-activated taurine transport. In cells in isotonic medium, pretreated then coincubated with 0.1 mM DIDS, the band 3-mediated transport of SO_4^{2-} and Cl^- was reduced to a low level. Exposure of these cells to a hypotonic medium containing 0.1 mM DIDS was followed by the activation of a Cl^- permeation pathway showing the same inhibitor sensitivity as swelling-activated taurine transport. The data are consistent with swelling-activated transport of taurine and Cl^- being via a common pathway. A comparison of the swelling-activated transport rates for taurine and Cl^- with those for several other solutes was consistent with the hypothesis that this pathway is an anion-selective channel, similar to those that mediate the volume-regulatory efflux of Cl^- and organic osmolytes from mammalian cells.

Key words: Taurine — Anion channel — Osmolyte — Volume regulation

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Introduction

Erythrocytes from a number of fish species have been shown to respond to osmotic cell swelling by undergoing a regulatory volume decrease (RVD). As in cells from higher vertebrates, this is achieved via the activation of membrane transport systems that allow the net efflux of both organic and inorganic solutes (and hence water) from the cells. In erythrocytes from elasmobranch and teleost fish, the major organic 'osmolyte' released in response to osmotic swelling is, as in many mammalian cells, taurine, a sulfonic amino acid (Fugelli & Thoroed, 1986; Fincham, Wolowyk & Young, 1987; Goldstein, Brill & Freund, 1990; Garcia-Romeu, Cossins & Motais, 1991; Jensen, 1995). Taurine is accumulated from the plasma (to intracellular concentrations of tens of millimolar) via a Na^+- and Cl^--dependent transporter, the mammalian form of which has been characterized at the molecular level (Uchida et al., 1992). The properties of the pathway that mediates taurine release following cell swelling are less well defined.

The pharmacological and kinetic properties of swelling-activated taurine transport in fish erythrocytes are very similar to those of volume-sensitive anion-selective channels in mammalian cells (Kirk, Ellory & Young, 1992; Goldstein & Davis, 1994). Recent evidence from a number of different cultured mammalian cell lines provides strong support for the hypothesis that such channels do indeed provide a route for the volume-regulatory efflux of taurine and other small organic osmolytes (e.g., Roy & Malo, 1992; Banderali & Roy, 1992; Strange et al., 1993; Jackson & Strange, 1993; Kirk & Kirk, 1993; Jackson, Morrison & Strange, 1994; Kirk & Kirk, 1994). However, although the pathway in fish erythrocytes has been shown to be permeable to a diverse range of electroneutral organic solutes (Kirk et al., 1992; Goldstein & Davis, 1994; Tiihonen, Nikinnaa & Lappivaara, 1995) there is little known about its ion-selectivity. The available data are consistent with the pathway having a sig-

nificant permeability to the monovalent cations choline (Garcia Romeu et al., 1991; Thoroed & Fugelli, 1994; Joyner & Kirk, 1994) and K^+ ($^{86}Rb^+$) (Bursell & Kirk, 1995). However, the anion permeability of this pathway remains to be established and quantified.

The measurement of Cl^- transport in erythrocytes from most vertebrate species is made technically difficult by the very high transport capacity of the constitutively active 'band 3' anion exchanger. The high rate of flux of Cl^- via this system makes it difficult to measure the transport of Cl^- via other pathways that might be present. In principle, this problem might be circumvented by the use of reagents that inhibit the anion exchanger. However, in practice, many of these compounds also inhibit swelling-activated solute transport (Goldstein et al., 1990; Goldstein & Brill, 1991; Garcia-Romeu et al., 1991; Motais, Guizouarn & Garcia-Romeu, 1991; Kirk et al., 1992; Goldstein & Davis, 1994). This has led several authors to propose a role for the band 3 protein in the swelling-activated response, either as a swelling-activated channel (Goldstein & Musch, 1994) or as a regulatory protein, coupling the activity of membrane transport systems to the volume-sensing mechanisms (Garcia-Romeu et al., 1991; Motais et al., 1991, 1992).

In this study, we have made a quantitative comparison of the effect of a range of inhibitors on the transport activity of band 3 with their effect on swelling-activated taurine transport. The two systems showed similar, but not identical pharmacology. The finding that the stilbene disulfonic acid, DIDS, was a more potent inhibitor of band 3-mediated anion exchange than of the volume-activated osmolyte channel enabled us to establish conditions under which it was possible to investigate the swelling-activated transport of Cl^- without interference from the anion exchanger. The resulting data are consistent with the swelling-activated osmolyte channel of fish erythrocytes being highly permeable to Cl^-, with a Cl^-/taurine permeability ratio similar to that measured (using electrophysiological methods) for swelling-activated anion-selective channels elsewhere.

Materials and Methods

Chemicals

[^{14}C]Taurine, [^{14}C]sorbitol, [^{14}C]choline and ^{86}RbCl were obtained from Du Pont-New England Nuclear; $^{36}Cl^-$ and $^{35}SO_4^{2-}$ were from Amersham International. Ouabain, *N*-ethylmaleimide (NEM), 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid disodium salt (DIDS), niflumic acid, furosemide and quinine hydrochloride were from Sigma Chemical. 5-Nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) was a gift from Prof. R. Greger (Physiologisches Institüt der Albert-Ludwigs-Universität, Freiburg, Germany).

Solutions

The basic iso-osmotic saline used for collecting, washing and storing eel erythrocytes contained (in mM): 150 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 15 3-[N-morpholino]propanesulfonic acid (MOPS) and 5 glucose, pH-adjusted to 7.5 using 1 M NaOH.

Unless specified otherwise, transport experiments were carried out with cells washed then resuspended in a high K^+ saline, containing (in mM): 155 KCl, 15 MOPS and 5 glucose, pH-adjusted to 7.5 with 1 M KOH. The high extracellular K^+ concentration served to reverse the normally outward K^+ concentration gradient across the erythrocyte membrane and thereby prevented the cells from undergoing RVD following osmotic swelling (Bursell & Kirk, 1995).

In experiments in which cells were subjected to osmotic swelling, a suspension of cells in an isotonic solution (300 ± 5 mOsm(kg H_2O)$^{-1}$) was normally diluted with an equal volume of a hypo-osmotic solution containing (in all experiments other than those giving rise to the Table) 5 mM KCl, 15 mM MOPS and 5 mM glucose (pH 7.5), to give a final osmolality of approximately 165 mOsm(kg H_2O)$^{-1}$.

Eel Erythrocytes

Common European eels (*Anguilla anguilla*) purchased from a commercial source were killed by decapitation followed by pithing of the brain. Blood was collected from the caudal vessels into ice-cold, heparinized, iso-osmotic saline (*as above*), then filtered through a polymer wool column to remove white cells and debris. The cells were centrifuged (1,500 × *g*, 5 min) and any remaining buffy coat removed by aspiration. The erythrocytes were washed three times in iso-osmotic saline, resuspended at a hematocrit of approximately 10% in the same solution (supplemented with 1% *w/v* bovine serum albumin), then left for a minimum of 3 hr, and in most cases overnight, to ensure that they were in a steady state before carrying out flux measurements (Cossins & Kilbey, 1989). In the 2–3 hr before experiments, the cells were regularly exposed to air to avoid changes in membrane transport activity arising from transition from the deoxygenated to the oxygenated state (Borgese, Motais & Garcia-Romeu, 1991; Nielsen, Lykkeboe & Cossins, 1992).

Transport Measurements

Throughout this study unidirectional influx rates were used as a measure of the activity of the transport systems of interest. $^{35}SO_4^{2-}$ influx into cells in isotonic medium provided a measure of the activity of the band 3 anion exchanger. Transport rates for taurine, Cl^-, sorbitol, choline and Rb^+ in cells in isotonic and hypotonic media were calculated from the uptake of [^{14}C]taurine, $^{36}Cl^-$, [^{14}C]sorbitol, [^{14}C]choline and $^{86}Rb^+$, respectively.

All flux measurements were carried out at 22°C using standard protocols similar to those described elsewhere (e.g., Joyner & Kirk, 1994). Briefly, cells were prewashed by repeated centrifugation and resuspension in the appropriate isotonic solution. In most experiments fluxes were commenced by combining an aliquot of cell suspension with an iso-osmotic or hypo-osmotic solution containing radiolabel (together with unlabeled substrate), to give a final osmolality of 300 or 165 mOsm(kg H_2O)$^{-1}$, respectively. The final hematocrit was ⩽ 5%. The activity of radiolabel used was 1 μCi/ml for $^{86}Rb^+$ and 0.5 μCi/ml for all other substrates. In experiments in which quantitative estimates were made of swelling-activated transport rates (Table), the cells were exposed to hypotonic media 2.5 min before the addition of isotope to ensure full activation of the system of interest before beginning the flux.

At a suitable time interval after combining cells and radiolabel, aliquots of the suspension were taken and layered over 0.5 ml of dibutylphthalate in microcentrifuge tubes that were then centrifuged (10,000 × *g*, 30 sec) to terminate the flux. In initial time-course ex-

periments aliquots of the suspension were sampled at regular intervals. In later experiments, initial influx rates were estimated from the amount of radioactivity accumulated within a fixed incubation period that fell within the initial, linear phase of the uptake time courses (10–15 min for $^{86}Rb^+$ and $^{36}Cl^-$ and 15–30 min for all other substrates).

Following centrifugation of the cells below the dibutylphthalate, the aqueous supernatant solution was removed by aspiration and the radioactivity remaining on the walls of the tube removed by rinsing the tubes four times with water. The dibutylphthalate was aspirated and the cell pellet lysed by mixing with 0.1% *v/v* Triton X-100 (0.5 ml) and deproteinized by the addition of 5% *w/v* trichloroacetic acid (0.5 ml), followed by centrifugation (10,000 × *g*, 10 min). The radioactivity in the supernatant solutions was measured by β-scintillation counting.

In all experiments, the amount of radiolabel trapped in the extracellular space within the cell pellets was estimated from *time zero* samples which were taken within a few seconds of combining the cells and the radiolabel.

Unidirectional influx rates were calculated in terms of iso-osmotic cell volumes which were estimated from the absorbance at 540 nm of cells diluted in 'Drabkin's reagent' (Dacie & Lewis, 1975). The theoretical absorbance of packed eel erythrocytes (i.e., of cells at 100% hematocrit) was estimated (from the absorbance at 540 nm of cells at known hematocrit diluted in Drabkin's reagent) as 187 ± 2 (mean ± SEM, $n = 3$) for a 1-cm light path.

Inhibitor Studies

DIDS, furosemide, niflumate, quinine and NPPB were added to cell suspensions as stock solutions in dimethylsulfoxide (DMSO). The DMSO concentration remained below 0.5% *v/v*. Unless specified otherwise, these inhibitors were added to the cell suspension together with radiolabeled substrate, at the time of commencing the flux. The sulfhydryl reagent NEM was dissolved (to a concentration of 300 mM) in hypo-osmotic solution and added, where appropriate (at a final concentration of 2 mM), to cells in isotonic, high-K^+ medium, 20 min before beginning the flux. The temperature throughout the 20-min pre-incubation was approximately 22°C and the hematocrit ⩽10%. The NEM remained present in the cell suspension throughout the flux period.

In experiments in which cells were pretreated with DIDS, the cells were washed then suspended in isotonic saline, then DIDS was added at a concentration of 0.1 mM. Following a 60-min incubation at 22°C, the cells were washed three times in (DIDS-free) saline, supplemented with 1% *w/v* bovine serum albumin (BSA), then twice more in BSA-free medium (modified from Motais et al., 1992).

Quantitative Comparison of Swelling-activated Transport Rates for Different Solutes

In one series of experiments (giving rise to the Table) the relative magnitudes of swelling-activated influx rates for five different solutes (Rb^+, choline, sorbitol, taurine and Cl^-) were compared. These experiments were carried out under conditions designed to minimize the flux through all pathways other than the swelling-activated system of interest. This was achieved by the use of appropriate inhibitors, together with a high extracellular choline concentration to saturate (and thereby minimize the uptake of [^{14}C]choline via) the constitutively active choline transporter (Joyner & Kirk, 1994).

Cells pretreated with DIDS (*as above*) were washed (×3) then resuspended (at a hematocrit ⩽ 10%) in an isotonic solution containing (in mM): 120 choline chloride, 5 NaCl, 15 MOPS and 5 glucose, together with sufficient sucrose (43) to give a final osmolality of 300 mOsm(kg $H_2O)^{-1}$. Ouabain was added at a concentration of 0.1 mM and the cells incubated for 10 min at 22°C to bring about inhibition of the Na^+/K^+ pump. A 342 μl aliquot of this suspension was then transferred to an Eppendorf tube and combined with a 90 μl aliquot of an isotonic (100 mM) $BaCl_2$ solution (made up in hypotonic solution containing 5 mM NaCl + 15 mM MOPS), together with 450 μl of either an isotonic or hypotonic solution containing (in mM): 150 NaCl + 15 MOPS + 5 glucose, or 5 NaCl + 15 MOPS + 5 glucose, respectively supplemented with 0.2 mM DIDS (± 0.6 mM NPPB). Influx commenced 2.5 min later with the addition to the suspension of an 18 μl aliquot of solution containing taurine, sorbitol and RbCl, each at a concentration of 75 mM, together with the radiolabeled form of one of the five substrates of interest. The final volume in the flux tube was 900 μl. The final concentrations of DIDS, Ba^{2+} and, where appropriate, NPPB, were (in mM): 0.1, 10 and 0.3, respectively. The final concentrations of the five transport substrates of interest were (in mM): 1.5 for Rb^+; 45.6 for choline; 1.5 for sorbitol; 1.5 for taurine; and, for Cl^-, 147 in the hypotonic samples and 72 in the isotonic samples.

For technical reasons it was not always possible for the fluxes of all five substrates to be measured within a single experiment. However all experiments included a measurement of [^{14}C]taurine influx, thus enabling in each case an estimate of the swelling-activated transport rate for the different substrates of interest relative to that for taurine.

To compare the relative rates of transport for substrates present at different extracellular concentrations the measured influx rates (mmol/(L RBC. hr)) were, for each substrate, divided by the extracellular substrate concentration (mM) to give an estimate of the flux through the system of interest for an extracellular substrate concentration of 1 mM (denoted by the symbol ΔR in the Table).

Results

Pharmacological Comparison of Band 3-Mediated SO_4^{2-} Transport and Swelling-activated Taurine Transport

A variety of anion transport blockers have been shown to inhibit swelling-activated solute transport in fish erythrocytes (Lauf, 1982; Goldstein et al., 1990; Goldstein & Brill, 1991; Garcia-Romeu et al., 1991; Motais et al., 1991; Kirk et al., 1992; Goldstein & Davis, 1994). Many of these compounds also inhibit anion exchange via the band 3 protein, prompting the suggestion that band 3 is involved in the volume-regulatory response (Goldstein et al., 1990; Goldstein & Brill, 1991; Motais et al., 1991, 1992; Goldstein & Musch, 1994). Figure 1 shows dose-response curves for the effect of six different reagents on the transport of SO_4^{2-} (a band 3 substrate) in cells in iso-osmotic medium, and on the swelling-activated transport of taurine in cells in hypo-osmotic solution. For NPPB, furosemide and niflumate the dose-response curves for the two different substrates were very similar. However, there was a significant difference between the sensitivity of the two pathways to NEM, quinine and DIDS. NEM inhibited swelling-activated taurine transport ($IC_{50} \simeq 1$ mM) but decreased band 3-mediated SO_4^{2-} transport only slightly at concentrations as high as 5 mM. Quinine inhibited swelling-activated taurine transport

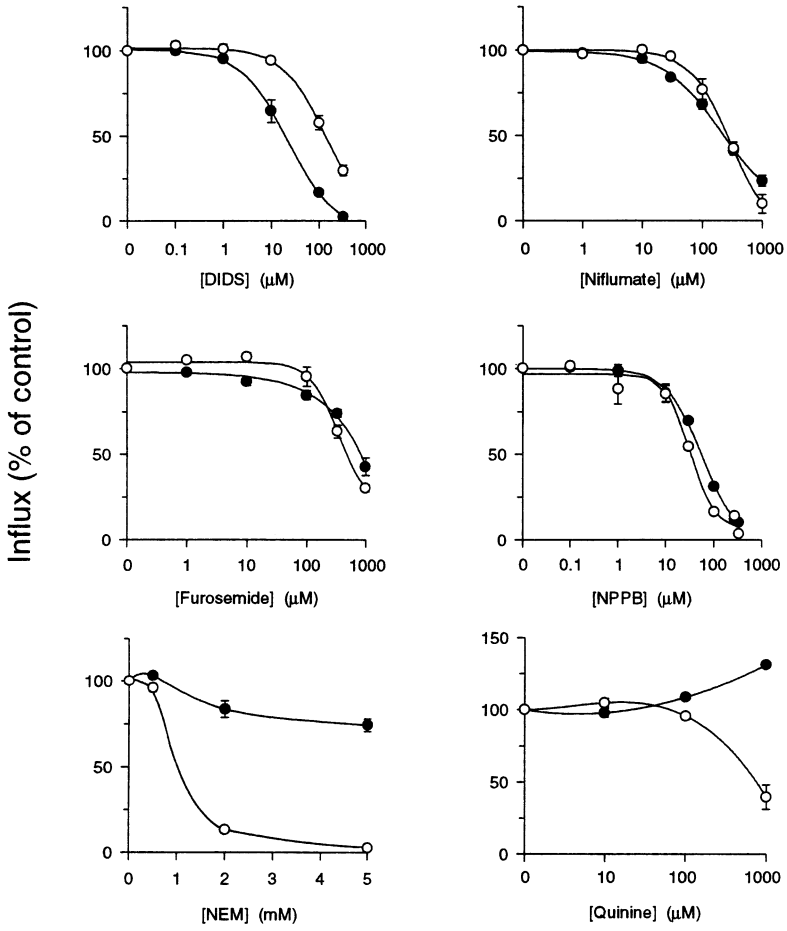

Fig. 1. Dose-response curves for the effect of inhibitors on band 3-mediated SO_4^{2-} transport in cells in an isotonic medium (filled circles) and on swelling-activated taurine transport (open circles). Swelling-activated taurine transport was calculated by subtracting the flux measured in isotonic medium (with an osmolality of 300 mOsm(kg $H_2O)^{-1}$) from that measured in hypotonic medium (with an osmolality of 165 mOsm(kg $H_2O)^{-1}$). The extracellular SO_4^{2-} concentration was 10 μM and the extracellular taurine concentration 1 mM. The fluxes are expressed as a percentage of those measured in the absence of inhibitors. The data are from the mean of three separate experiments, each performed on blood from different eels, and are shown ± SEM.

(IC_{50} < 1 mM) but *increased* the rate of SO_4^{2-} transport. Conversely, DIDs was a more potent inhibitor of band 3-mediated SO_4^{2-} transport than of swelling-activated amino acid transport.

The effect of DIDS on the two transport activities was investigated in more detail and the results are shown in Fig. 2. In cells pretreated with 0.1 mM DIDS, then washed and resuspended in DIDS-free medium, band 3-mediated SO_4^{2-} transport was reduced by approximately 85%, consistent with an irreversible interaction between DIDS and the transporter. Exposure to 0.1 mM DIDS throughout the (28 min) flux period, without pretreatment, was similarly effective. In cells pretreated with DIDS *and* exposed to DIDS throughout the flux, band 3-mediated SO_4^{2-} transport was reduced by over 95%.

Swelling-activated taurine transport showed a quite different pattern of inhibition by DIDS (Fig. 2). Pretreatment of cells with DIDS (followed by its removal before beginning the flux) had little effect on swelling-activated taurine transport. In cells exposed to 0.1 mM DIDS during the flux period (without pretreatment), swelling-activated taurine transport was reduced by only 30%. A similar level of inhibition was seen in cells exposed to DIDS both before and during the flux.

Swelling-activated Cl^- Transport

The band 3 anion exchanger serves as a high capacity Cl^- transporter and the high rate of flux of Cl^- via this system makes it difficult to measure the transport of Cl^- via other pathways that might be present. However, the data of Fig. 2 indicate that in cells pretreated with and then resuspended in the presence of 0.1 mM DIDS, the band 3 anion exchanger was inhibited almost fully, whereas the swelling-activated taurine transport pathway remained operative. We therefore took advantage of these conditions to investigate the permeability of the swelling-activated pathway to Cl^-.

Figure 3 shows time courses for the transport of Cl^- into eel erythrocytes that had been pretreated with 0.1 mM DIDS and then resuspended (at $t = 0$) in either iso-osmotic or hypo-osmotic media containing 0.1 mM DIDS. For cells in isotonic solution, there was no significant Cl^- uptake over a 10-min period, consistent with

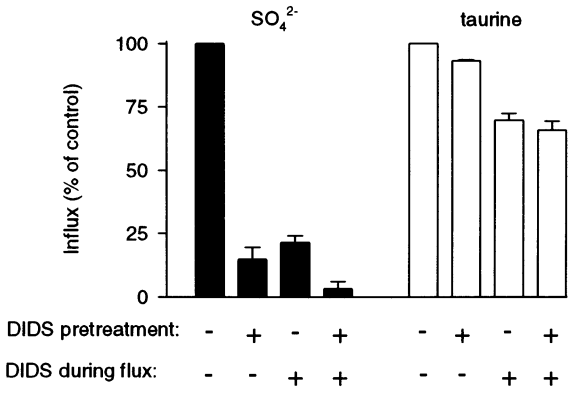

Fig. 2. Effect of DIDS on band 3-mediated SO_4^{2-} transport in cells in an isotonic medium (filled bars) and on swelling-activated taurine transport (open bars). Cells were incubated for 1 hr in the presence or absence of 0.1 mM DIDS, washed three times in saline supplemented with 1% *w/v* BSA, then twice more in saline without BSA. Fluxes were measured in the presence or absence of 0.1 mM DIDS (added at the time of combining cells and radioisotope). The extracellular SO_4^{2-} concentration was 10 μM and the taurine concentration 1 mM. Swelling-activated taurine transport was calculated by subtracting the flux measured in isotonic medium (with an osmolality of 300 mOsm(kg $H_2O)^{-1}$) from that measured in hypotonic medium (with an osmolality of 165 mOsm(kg $H_2O)^{-1}$). The fluxes are expressed as a percentage of those measured in cells not treated with DIDS. The data are from the mean of three separate experiments performed on blood from different eels and are shown ± SEM.

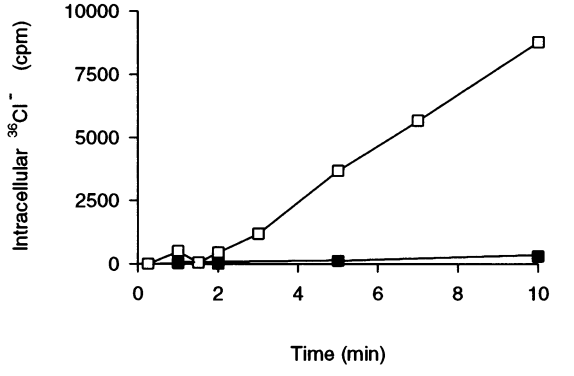

Fig. 3. Time courses for influx of Cl^- into eelerythrocytes suspended (at $t = 0$) in isotonic (300 mOsm(kg$H_2O)^{-1}$); filled squares) or hypotonic (165mOsm(kg $H_2O)^{-1}$; open squares) media. The cells were pretreated and incubated throughout the flux period with 0.1mM DIDS (as in Fig. 2) in order to inhibit the flux ofCl^- via the constitutively active band 3 anion exchanger.Results are from one experiment and are representative of data obtainedin three similar experiments on blood from different eels.

full inhibition of band 3. For cells swollen by exposure (at $t = 0$) to hypotonic solution, there was a short (1–2 min) lag, after which there was a steady influx of Cl^-.

Figure 4 shows a comparison of the effects of inhibitors on the swelling-activated transport of taurine and

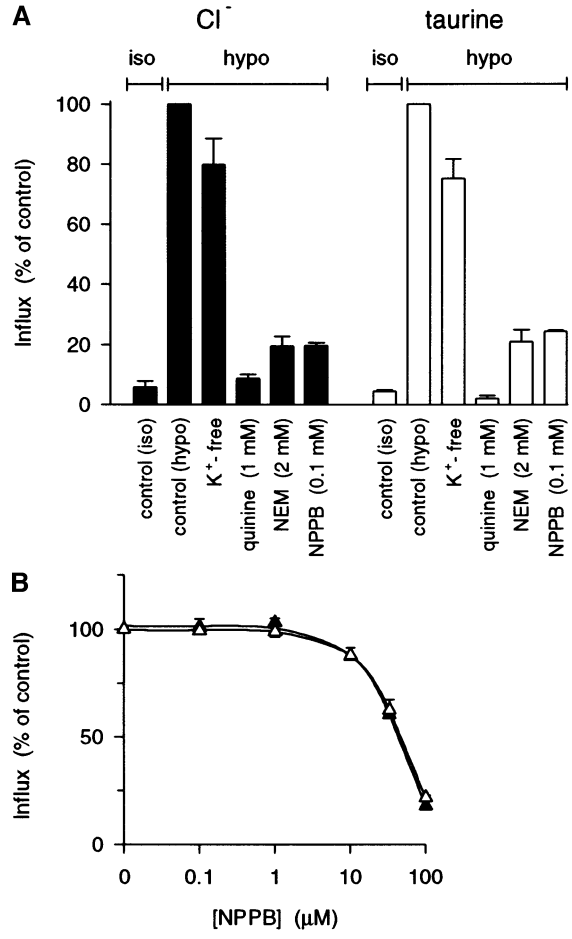

Fig. 4. Comparison of the effect of inhibitors on the swelling-activated transport of taurine (open bars/symbols) and Cl^+ (filled bars/symbols). The cells were pretreated and incubated throughout the flux period with 0.1 mM DIDS (as in Fig. 2) in order to inhibit the band 3 anion exchanger. **(A)** Shows the effects of single concentrations of quinine, NEM and NPPB, as well as the effect of iso-osmotic replacement of extracellular K^+ with Na^+. The data are averaged from three experiments, each on blood from different eels, and are shown ± SEM. **(B)** Shows dose-response curves for the effect of NPPB on the swelling-activated transport of taurine and Cl^- (calculated by subtracting the fluxes measured under isotonic conditions from those measured in cells in hypotonic media). The data are averaged from two sets of duplicate experiments carried out on blood from two different eels.

Cl^- (in cells treated both before and during the flux with 0.1 mM DIDS). The swelling-activated transport of these two solutes was inhibited to the same extent by (in mM) 1 quinine, 2 NEM and 0.1 NPPB (Fig. 4*A*). The dose-response curves for the effect of NPPB on the swelling-activated transport of Cl^- and taurine were superimposable (Fig. 4*B*) and the swelling-activated transport of both substrates showed the same slight decrease on replacement of K^+ with Na^+ in the extracellular solution (Fig. 4*A*). These data are consistent with swelling-

Table. Relative magnitudes of swelling-activated influx rates for Rb^+, choline, sorbitol, taurine and Cl^- in eel erythrocytes

Substrate (X)	± NPPB	R(*iso*)	R(*hypo*)	ΔR	$\Delta R_X/\Delta R_{taurine}$
		(mmol/(L RBC · hr · mM))			
Rb^+	−	0.03 ± 0.03 (5)	0.36 ± 0.06 (5)	0.33 ± 0.05 (5)	0.47 ± 0.06
	+		0.12 ± 0.02 (3)		
Choline	−	0.09 ± 0.01 (4)	0.22 ± 0.04 (4)	0.13 ± 0.04 (4)	0.21 ± 0.02
	+		0.11 ± 0.03 (4)		
Sorbitol	−	0.12 ± 0.02 (4)	0.33 ± 0.05 (4)	0.21 ± 0.05 (4)	0.34 ± 0.04
	+		0.15 ± 0.04 (4)		
Taurine	−	0.13 ± 0.02 (6)	0.90 ± 0.16 (6)	0.77 ± 0.15 (6)	1
	+		0.17 ± 0.09 (4)		
Cl^-	−	0.10 ± 0.02 (3)	1.98 ± 0.28 (3)	1.88 ± 0.27 (3)	3.81 ± 0.24
	+		0.08 ± 0.02 (3)		

The symbol R denotes the measured influx rate (mmol/(L RBC · hr)) divided by the extracellular substrate concentration (mM). R(*iso*) refers to fluxes measured under isotonic conditions and R(*hypo*) to fluxes measured under hypotonic conditions (in the presence or absence of 0.3 mM NPPB). ΔR, the difference between them, is equivalent to the influx of substrate via the swelling-activated pathway of interest for an extracellular substrate concentration of 1 mM. The experiments were carried out as described in Materials and Methods. The osmolality in the isotonic cell suspensions was 300 mOsm(kg $H_2O)^{-1}$ and that in the hypotonic suspensions 165 mOsm(kg $H_2O)^{-1}$. The numbers in brackets show the number of experiments, each on blood from a different eel. The $\Delta R_X/\Delta R_{taurine}$ values were averaged from those estimated in experiments in which the fluxes of the different substrates (X) were paired with those for taurine. The significant variability between experiments in the magnitude of the swelling-activated fluxes prevents meaningful comparisons between the magnitudes of the fluxes measured for the different substrates in unpaired experiments.

activated Cl^- and taurine transport being via a common pathway.

Comparison of the Rates of Swelling-activated Transport for Different Solutes

Data from previous studies of swelling-activated solute transport in fish erythrocytes are consistent with the hypothesis that swelling-activated taurine transport is via a broad-specificity channel that accommodates a wide range of solutes including amino acids, polyols and nucleosides (Kirk et al., 1992; Haynes & Goldstein, 1993; Goldstein & Davis, 1994; Thoroed & Fugelli, 1994) as well as the monovalent cations choline (Garcia-Romeu et al., 1991; Joyner & Kirk, 1994; Thoroed & Fugelli, 1994) and $Rb^+(K^+)$ (Bursell & Kirk, 1995). The data of Figs. 3 and 4 are consistent with Cl^- permeating this channel and it was therefore of interest to compare its rate of transport with that of other permeant solutes.

The Table shows results averaged from a series of paired experiments comparing the rate of influx of taurine, sorbitol, choline, Rb^+ and Cl^- in eel erythrocytes suspended in isotonic media and hypotonic media (± NPPB). These experiments were carried out under conditions designed to minimize the flux through all pathways other than the swelling-activated system of interest. As in the experiments of Figs. 2–4 the cells were pretreated with 0.1 mM DIDS and this reagent was also included in the flux suspension to inhibit band 3-mediated anion-exchange as well as the swelling-activated KCl cotransporter (Delpire & Lauf, 1992; Bursell & Kirk, 1995). The cells were preincubated for 10 min with 0.1 mM oubain to inhibit the Na^+/K^+ pump and 10 mM $BaCl_2$ was included in the suspension to inhibit any possible contribution of Ba^{2+}-sensitive K^+ channels to the measured cation fluxes (Nonnotte & Truchot, 1992). Uptake of [^{14}C]choline via the high-capacity but saturable choline transporter in these cells was minimized by the use of a saturating concentration of this substrate (45.6 mM). Under these conditions, the swelling-activated (NPPB-sensitive) transport of the five different solutes tested was consistently in the order choline < Rb^+ < sorbitol < taurine < Cl^- (Table).

Discussion

Swelling-activated Taurine Transport is Pharmacologically Distinct from Band 3-Mediated SO_4^{2-} Transport

Fish erythrocytes, like many mammalian cells, respond to osmotic swelling by releasing taurine. This process plays a central role in the volume-regulatory response. The pathway that mediates swelling-activated taurine release is inhibited by a range of anion transport blockers. This, in combination with kinetic data and the broad substrate selectivity of the pathway, has led to the proposal that it is an anion-selective channel (Kirk et al., 1992), similar to swelling-activated anion channels that have been described in many other cell types (e.g., Jackson & Strange, 1993). However, these compounds also inhibit the erythrocyte band 3 anion exchange protein, leading some investigators to suggest that their effect on

volume-activated transport may be due to their interaction with band 3, and that this protein may therefore function either as a channel (Goldstein & Musch, 1994) or as a channel regulator (Motais et al., 1991, 1992).

In this study, we have made a quantitative comparison of the effect of inhibitors on swelling-activated taurine release and their effect on band 3-mediated SO_4^{2-} transport (Fig. 1). Furosemide, niflumate and NPPB each inhibited the two systems with similar potency. However, NEM and quinine were effective inhibitors of swelling-activated taurine transport while causing little or no inhibition of band 3-mediated SO_4^{2-} exchange within the concentration ranges tested. These results are similar to those reported by Goldstein and Davis (1994), who found that in skate erythrocytes quinine, arachidonic acid and pyridoxal-5-phosphate were more potent inhibitors of swelling-activated taurine transport than of band 3-mediated SO_4^{2-} exchange. These data do not rule out the possibility of a role for band 3 in swelling-activated solute transport. However they do indicate that a functional anion exchanger is not, by itself, sufficient for the cell to mount a volume-regulatory response.

In contrast to the other inhibitors tested, DIDS was a more effective inhibitor of band 3-mediated anion exchange than of the swelling-activated osmolyte pathway. Again, this does not preclude a role for band 3 in swelling-activated osmolyte transport. However, it would suggest that the interaction by which DIDS blocks anion exchange is not the same interaction by which it inhibits the swelling-activated pathway. This conclusion is borne out by the data of Fig. 2 which show that preincubation of cells with DIDS caused irreversible inhibition of band 3-mediated anion exchange, while having little effect on the pathway activated by subsequent cell swelling.

Swelling-activated Cl^- Transport is Pharmacologically Indistinguishable from Swelling-activated Taurine Transport

The finding that DIDS was a more potent inhibitor of anion exchange than of the volume-regulatory response allowed us to investigate swelling-activated Cl^- transport under conditions where the contribution from the anion exchanger to the uptake of radiolabel was eliminated. Exposure of cells to a hypo-osmotic medium was followed (after a short lag) by the activation of a Cl^- permeation mechanism. The swelling-activated Cl^- flux showed the same sensitivity to quinine, NEM and NPPB as swelling-activated taurine transport (Fig. 4*A* and *B*), consistent with Cl^- and taurine sharing a common pathway. The possibility that a significant component of swelling-activated Cl^- transport was via the volume-sensitive KCl cotransporter can be ruled out on the following grounds: (i) the experiments were carried out in the presence of 0.1 mM DIDS which is sufficient to bring about full inhibition of KCl cotransport in these cells (Delpire & Lauf, 1992; Bursell & Kirk, 1995); (ii) swelling-activated Cl^- influx was diminished only slightly (and to the same extent as swelling-activated taurine influx) following the complete removal of K^+ from the extracellular solution; (iii) swelling-activated Cl^- transport was inhibited by 2 mM NEM, whereas KCl cotransport is stimulated by this concentration of the sulfhydryl reagent (Bursell & Kirk, 1995) (though it should be noted that high concentrations of NEM do inhibit KCl cotransport in a temperature-dependent manner (Lauf & Adragna, 1995)).

Comparison of the Swelling-activated Osmolyte Channel in Eel Erythrocytes with the Volume-sensitive Organic Osmolyte/Anion Channel (VSOAC) of Mammalian Cells

There is now substantial evidence from a number of mammalian cell types that the volume-regulatory efflux of organic osmolytes such as taurine, and the polyols sorbitol and myo-inositol, is via a swelling-activated, outwardly rectifying, anion-selective channel which K. Strange and coworkers have termed VSOAC, for 'Volume-Sensitive Organic osmolyte/Anion Channel' (Jackson et al., 1994). The results of the present study are consistent with a similar channel mediating the swelling-activated transport of organic osmolytes in fish erythrocytes.

Like the pathway in fish erythrocytes, the channel in mammalian cells is blocked by a range of anion transport inhibitors including NPPB, DIDS, quinine and niflumate. Electrophysiological estimates of $P_{\text{taurine}^-}/P_{Cl^-}$ range from 0.20 in rat glioma C6 cells (Jackson & Strange, 1993) to 0.75 in MDCK cells (Banderali & Roy, 1992). The estimate in this study (Table) of an apparent $P_{\text{taurine}}/P_{Cl^-}$ ratio of 0.26 (equating $\Delta R_{\text{taurine}}/\Delta R_{Cl^-}$ with $P_{\text{taurine}}/P_{Cl^-}$ and recognizing that $\Delta R_{\text{taurine}}$ is a measure of the swelling-activated influx of the combined anionic and zwitterionic forms of the amino acid) falls within this range. In a recent study of swelling-activated solute transport in bovine articular chondrocytes, the swelling-activated transport rate for sorbitol was 0.66 that for taurine (Hall, 1995), whereas in HeLa cells swelling-activated (NPPB-sensitive) sorbitol transport was 0.48 the rate of swelling-activated taurine transport (Hall et al., 1995). These values are of similar magnitude to the $\Delta R_{\text{sorbitol}}/\Delta R_{\text{taurine}}$ value of 0.34 estimated in the present study.

One significant difference between the reported characteristics of VSOAC in mammalian cells and the swelling-activated pathway in eel erythrocytes is in the apparent cation permeability of the two pathways. Electrophysiological studies of the mammalian system indi-

cate a (conductive) monovalent cation permeability of 0.02–0.04 times the Cl^- permeability (Jackson & Strange, 1993). In eel erythrocytes, the choline permeation rate was 0.06 times that of Cl^-, while the Rb^+ permeation rate was even higher, at 0.12 times that of Cl^- (Table). It is possible that the channel in fish cells does have a higher conductive cation permeability than its mammalian counterpart. Data from a recent electrophysiological study in which it was shown that a swelling-activated (taurine-permeable) anion-selective channel in skate hepatocytes has a relatively high cation permeability is consistent with this view (Jackson et al., 1995). Alternatively (or perhaps additionally) it is possible that monovalent cations permeate the pathway in the form of electroneutral cation:anion pairs that would not be detected by electrophysiological techniques. The fact that the pathway in eel erythrocytes is able to accommodate solutes as large as nucleosides (Kirk et al., 1992) would suggest that its diameter is sufficient to accommodate a simple ion pair, though whether this mechanism does actually contribute significantly to the swelling-activated transport of monovalent cations remains to be established.

Conclusion

In summary, the results of the present study indicate that the swelling-activated osmolyte pathway in eel erythrocytes may be distinguished pharmacologically from the constitutively active band 3 anion exchanger. The data do not preclude a role for band 3 in the volume-regulatory response but do suggest that, in these cells at least, the mechanism by which anion transport blockers such as DIDS inhibit swelling-activated osmolyte transport is different from that by which they inhibit band 3-mediated anion exchange. A quantitative comparison of swelling-activated transport rates for a series of solutes provides the first estimate of the relative Cl^- permeability (and hence the anion-selectivity) of the swelling-activated osmolyte pathway in fish erythrocytes. These data provide further support for the hypothesis that the swelling-activated organic osmolyte pathway in these cells is a volume-sensitive, anion-selective channel with characteristics very similar to those of the mammalian channel, VSOAC.

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