J. Membrane Biol. 158, 1–16 (1997)

The Journal of
Membrane
Biology

Topical Review

Swelling-activated Organic Osmolyte Channels

K. Kirk
Division of Biochemistry and Molecular Biology, Faculty of Science, Australian National University, Canberra A.C.T. 0200, Australia

Received: 9 December 1996/Revised: 25 March 1997

Introduction

Most cells have in their cytosols substantial (i.e., ⩾millimolar) concentrations of low molecular weight organic solutes which, together, make a significant contribution to the total intracellular osmolality and which are known collectively as 'organic osmolytes'. The solutes fulfilling this role fall, in most cases, into one of three different classes: amino acids (e.g., the α-amino acids alanine and proline, and the β-amino acids taurine and β-alanine), polyols (e.g., sorbitol and *myo*-inositol), and methylamines (e.g., betaine and glycerophosphoryl choline). Such compounds are either synthesized within the cell or taken up from the extracellular medium via accumulative ('active') transport systems. In contrast to inorganic ions which, at high concentrations, destabilize protein structure, these organic solutes exert a stabilizing influence on intracellular proteins and, for this reason, are termed 'compatible solutes' (Yancey, 1994).

The identity and intracellular concentrations of the major organic osmolytes vary between cell-types, as well as with the conditions to which the cells are exposed. Intracellular levels of these compounds increase markedly in response to cell shrinkage. Conversely, a common phenomenon that has been demonstrated for a wide range of cells is that such compounds are released in response to an acute increase in the cell volume, as occurs, for example, following a sudden decrease in the extracellular osmolality. Their loss from the cell is accompanied by a net efflux of water and this process thereby serves as part of the cell's volume-regulatory response.

From studies of swelling-activated organic osmolyte transport in cells from a diverse range of organisms it has emerged that the transport pathways involved share a number of functional characteristics. There is increasing evidence that these pathways are, in many cases and perhaps in general, channels that have a significant permeability to a wide variety of both charged and uncharged solutes. The purpose of this review is to summarize what is currently known about these pathways in eukaryotic cells. The focus is on the properties of the pathways themselves. The mechanisms underlying their volume-sensitivity and the regulatory processes involved in their activation are not considered in any detail. Current ideas concerning the functional and molecular characteristics of the pathways are discussed. However, before turning to these issues it is relevant to consider the relative contribution that these pathways make to the process of 'regulatory volume decrease' (RVD) in different cell and tissue types.

The Relative Contribution of Organic Osmolyte Loss to RVD varies Between Cell-Types and the Conditions to which the Cells are Exposed

Protozoa

In unicellular protozoa, arguably the most primitive extant eukaryotes, amino acids account for a large fraction of the total intracellular osmolality and they are the major solutes released by these cells in response to acute hypotonic stress. A recent analysis of the amino acid contents of three parasitic protozoa—*Giardia intestina-*

Correspondence to: K. Kirk

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

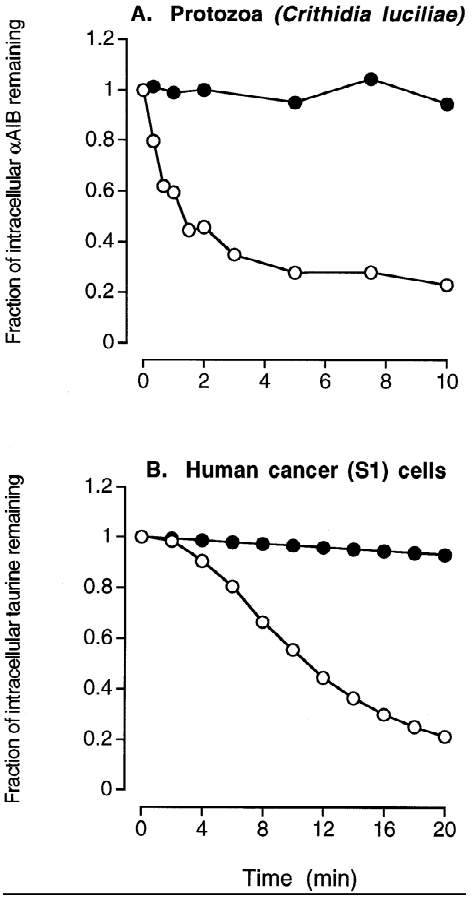

Fig. 1. Swelling-activated efflux of amino acids from (*A*) the protozoan parasite, *Crithidia luciliae* and (*B*) human lung cancer (S1) cells. (*A*) Shows time courses for the loss from *C. luciliae* of α-aminoisobutyrate (αAIB), a non-metabolizable analogue of alanine (the major organic osmolyte in this and many other protozoan species), following exposure of the cells (at $t = 0$) to either isotonic (300 mOsm/kg H_2O; ●) or hypotonic (100 mOsm/kg H_2O; ○) conditions. (Adapted from Bursell et al., 1996). (*B*) Shows time courses for the loss from human lung cancer cells of taurine, a common organic osmolyte in vertebrate cells, on exposure of the cells (at $t = 0$) to either isotonic (300 mOsm/kg H_2O; ●) or hypotonic (200 mOsm/kg H_2O; ○) conditions. (Adapted from Kirk & Kirk, 1993.)

lis, Trichomonas vaginalis and *Crithidia luciliae*—found the total intracellular amino acid concentrations (under standard culture conditions) to be 116, 57 and 148 mM, respectively (Knodler et al., 1994). The amino acid profiles vary between the different organisms; however in each case alanine is a major component of the free amino acid pool. Exposure of *G. intestinalis* (Park et al., 1995) and *C. lucililae* (Bursell et al., 1996), as well as other protozoa (Geoffrion & Larochelle, 1984; Darling et al., 1990; Vieira et al., 1996), to hypotonic conditions is followed by a rapid release of amino acids (predominantly alanine; Fig. 1*A*). In *C. luciliae*, a flagellated parasite that inhabits the gut of insects, this accounts for more than half of the volume-regulatory response following a two-thirds reduction in the extracellular osmolality (Bursell et al., 1996). In the soil-dwelling protozoan *Acanthamoeba castellanii* the loss of amino acids in the hour following a decrease in the extracellular osmolality from 240 to 40 mOsm/kg H_2O exceeds that of inorganic ions by almost threefold (Geoffrion & Larochelle, 1984). Similarly, in the promastigote (insect-dwelling) form of the human pathogen, *Leishmania donovani*, the loss of amino acids in the minutes immediately following a 50% decrease in the ambient osmolality exceeds the loss of K^+ by some tenfold (Darling et al., 1990; Blum, 1992).

Marine Molluscs

The tissues of euryhaline molluscs contain high concentrations of organic osmolytes, most notably taurine (a sulfonic amino acid that is not incorporated into proteins and which, with a pK_2 of 8.82, is present predominantly as an electroneutral zwitterion under physiological conditions), glycine betaine (a trimethylamine), and other amino acids (Amende & Pierce, 1980; Neufeld & Wright, 1996*b*). There have been numerous studies demonstrating the loss of such compounds from tissues following a reduction in the external osmolality. However, in many cases the proportion of the total intracellular osmolyte pool that is lost from the tissue is relatively small and there is little, if any RVD, at least in the short-term (Neufeld & Wright, 1996*a,b*). One apparent exception to this is ventricular tissue from the bivalve *Geukensia demisa* which releases a significant fraction of its amino acid pool under hypotonic conditions (Pierce & Greenberg, 1972, 1973; Deaton, 1994; Neufeld & Wright, 1996*b*). Another is blood cells from the bivalve *Noetia ponderosa* in which osmotic swelling is followed by a rapid release of KCl followed by a slower and smaller loss of taurine, with both contributing significantly to the overall (partial) RVD (Smith & Pierce, 1987).

Lower Vertebrates

Fish erythrocytes have proven to be extremely useful as a model system in which to study mechanisms of cell volume regulation. Hagfish and lampreys are the two surviving members of the agnathans and are the most ancient extant vertebrates species. Hagfish erythrocytes contain very high concentrations of amino acids (approximately 100 mM under physiological conditions; Fincham et al., 1990) but do not regulate their volume in response to osmotic swelling (Brill et al., 1992; Nikinmaa et al., 1993). By contrast lamprey erythrocytes do undergo partial RVD in hypotonic media. This process involves the activation of K^+ and Cl^- efflux mechanisms, without any increase in the membrane permeability to

the amino acid taurine (Brill et al., 1992; Virkki & Nikinmaa et al., 1993). Erythrocytes from elasmobranchs (skate) have very high levels of amino acids in their cytosol, with taurine and β-alanine as the major components of the free amino acid pool (Boyd et al., 1977). These are released from the cell in response to osmotic swelling (Haynes & Goldstein, 1993). Under the same conditions there is also increased efflux of K^+; however the rate of K^+ loss from skate red cells following a 50% reduction of the extracellular osmolality is tenfold less than the rate of taurine loss and in these cells the RVD is due predominantly to the efflux of amino acids (Dickman et al., 1990).

Erythrocytes from the evolutionarily less ancient teleost fish species also contain high concentrations of amino acids. Taurine is present at a cytosolic concentration typically in the range 25–55 mM (e.g., Fincham et al., 1987; Garcia-Romeu et al., 1991). Osmotic swelling of teleost erythrocytes is followed by a rapid release of taurine and other amino acids. In trout erythrocytes swollen by reduction of the extracellular osmolality by one third the loss of free amino acids accounts for approximately half of the total RVD, the remainder being due to the loss of K^+ and Cl^- (Garcia Romeu et al., 1991). Similar results have been obtained with erythrocytes from flounder (Fugelli & Thoroed, 1986; Nonnote & Truchcote, 1992) and from carp (Jensen, 1995).

Mammalian Cells

Many mammalian cells contain high cytosolic concentrations of organic solutes. Taurine is commonly the predominant intracellular organic osmolyte, with concentrations as high as 60 mM in some cell-types (Huxtable, 1992). Some mammalian cells also contain significant concentrations of polyols such as sorbitol and *myo*-inositol, with the intracellular levels of these solutes increasing on prolonged exposure of the cells to hypertonic conditions. For example, in rat brain (C6) glioma cells grown in standard culture medium (of osmolality 285-290 mOsm/kg H_2O) the intracellular *myo*-inositol concentration is in the range 26–40 mM; acclimation of these cells to hypertonic medium (of osmolality 435–445 mOsm/kg H_2O) for two days results in the intracellular *myo*-inositol concentration increasing to 100–150 mM (Strange & Morrison, 1992). Similarly, cells from the renal medulla which, in vivo, are exposed to high (and variable) extracellular NaCl concentrations during the process of urine production have high intracellular concentrations of sorbitol and *myo*-inositol (Kinne et al., 1993; Burg, 1994). When renal medullary cells are grown in standard culture medium (of osmolality ~315 mOsm/kg H_2O) the concentrations of these solutes are relatively low; however they increase markedly (over a period of several days) on exposure of the cells to media made hypertonic by the addition of NaCl (e.g., Nakanishi et al., 1988).

Swelling-activated release of both organic and inorganic solutes has been reported from a very wide range of mammalian cell and tissue types. However, there have been relatively few quantitative analyses of the relative contributions of the different classes of solute to the overall RVD. In a number of studies of the response of mammalian cells to a decrease in the osmolality the efflux of K^+ has been shown to exceed the efflux of Cl^- and, in some cases, the total Cl^- content of the cell. It has therefore been postulated that there is a significant efflux of (in most cases unidentified) organic anions, counterbalancing the charge loss associated with K^+ efflux. This is the case in HL-60 (human promyelocytic leukemia) cells (Hallows & Knauf, 1994), as well as in MDCK (canine kidney) cells (Roy & Sauvé, 1987), Ehrlich ascites tumor cells (Hoffmann et al., 1984) and lymphocytes (Deutsch & Lee, 1988). The anions involved have been postulated to include the anionic amino acids glutamate and aspartate, together with metabolites such as lactate.

In an early study of Ehrlich ascites tumor cells in medium in which the osmolality was reduced by 25% it was estimated that approximately one third of the RVD undergone by the cells was due to the efflux of amino acids (predominantly taurine) and two-thirds to the release of inorganic ions (Hoffmann & Hendil, 1976). In a study of the volume-regulatory response of MDCK cells swollen by a >50% decrease in the extracellular osmolality the total amino acid loss was similar to the total Cl^- loss and approximately half the total loss of K^+ (Roy & Sauvé, 1987). The significant role played by amino acids in general and taurine in particular in the RVD response of this cell type was confirmed by Pasantes Morales and colleagues (Sánchez Olea et al., 1991). The same group also demonstrated a significant role for amino acid release in the volume-regulatory response of mouse astrocytes, with the loss of taurine accounting for approximately one quarter of the RVD undergone by cells exposed to a medium having half the normal osmolality (Pasantes Morales & Schousboe, 1988). However, in most other studies of mammalian cells the role of organic osmolytes in the volume-regulatory response is less clear-cut. There have been numerous demonstrations of an increased flux of radiolabeled taurine and other amino acids across the plasma membrane of different mammalian cell-types following exposure to hypotonic media (as in Fig. 1*B*). However, in most such studies it is unclear whether the net amino acid loss is sufficient to make a significant contribution to the total RVD. In recent studies quantifying the magnitude of the taurine and/or amino acid loss from perfused rat liver (Brand et al., 1994), bovine articular chondrocytes (Hall, 1995) and rat brain glial (C6) cells (Mountian et al.,

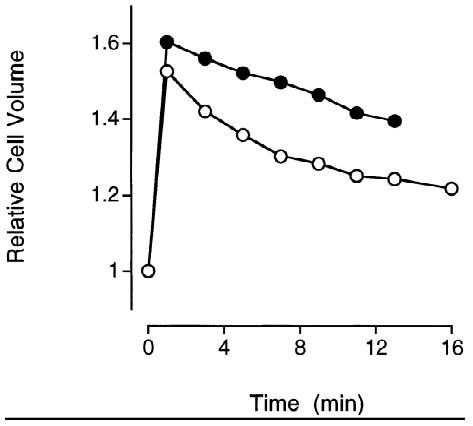

Fig. 2. Decreased efficacy of the RVD response in taurine-deficient rat cerebellar astrocytes. Cells preincubated in either standard culture medium, supplemented with 10% v/v fetal calf serum (○) or a chemically defined medium lacking taurine (●) were swollen by reducing the extracellular osmolality (at $t = 0$) from 300 to 150 mOsm/kg H_2O. Cell volumes are expressed relative to those under isotonic conditions. (Adapted from Morán et al. (1994), with permission.)

1996) it was concluded that in each case the contribution of this process to the total RVD was relatively small in comparison to the contribution of inorganic ions. Arguments have been made for a significant role for the efflux of taurine in the RVD response of rat brain (Law, 1994*a*,*b*) and lactating rat mammary gland (Shennan et al., 1993; Shennan et al., 1994). However in each case the magnitude of this flux, relative to that of inorganic solutes was not determined.

In cells acclimated to hyperosmotic conditions and containing high cytosolic levels of sorbitol and/or *myo*-inositol re-exposure to iso-osmotic conditions results in cell swelling, to which the cells respond by the rapid release of these polyols (e.g., Siebens & Spring, 1989; Kinne et al., 1993; Strange & Morrison, 1992). This process undoubtedly makes a significant contribution to the volume-regulatory response under these conditions. However, the magnitude of this contribution relative to that made by the efflux of inorganic ions has not been quantified.

Before concluding this section it is perhaps worth sounding a cautionary note with regard to evaluating the relative importance of different RVD mechanisms in different cell-types. In assessing the volume-regulatory capability of cells cultured in vitro it is relevant to consider the conditions to which the cells are exposed prior to experimentation. Cells derive their high cytoplasmic levels of taurine (as well as some other organic osmolytes) almost entirely via their uptake from outside the cell, and the maintenance of normal intracellular taurine levels is usually reliant upon there being normal taurine concentrations in the external medium (e.g., Sánchez Olea et al., 1991). Most standard culture media do not contain taurine, and although it is normal practice to supplement media with 10% *v/v* serum this still limits the availability of extracellular taurine to below that which may be required for maintenance of normal intracellular levels. In view of this, the physiological relevance of studies in which cells cultured in vitro and exposed to hypotonic media are shown to undergo channel activation but not undergo RVD (e.g., Altenberg et al., 1994) or in which organic osmolyte efflux is found to make a negligible contribution to the observed RVD response of cultured cells (e.g., Mountian et al., 1996) is questionable. This point is illustrated in Fig. 2, taken from a study by Morán et al. (1994) in which it was demonstrated that the volume-regulatory capability of cultured astrocytes varies with the intracellular taurine concentration and is diminished in cells cultured in taurine-depleted media. Similar considerations apply to experiments with isolated cells or tissues: exposure to media containing subphysiological levels of important osmolytes for prolonged periods prior to experimentation may deprive the cells of the resources required for their normal volume regulatory response.

In summary, the contribution of organic osmolyte efflux to the RVD response of eukaryotic cells swollen in hypotonic media ranges from being relatively major (in the case of protozoa and other invertebrates, lower vertebrates, and some mammalian cells) to relatively minor (in the case of other mammalian cells). However before discounting a significant role for this process in a particular cell-type it is relevant to consider whether the conditions to which the cells were exposed prior to experimentation are sufficient to ensure that the cells are equipped with their normal complement and concentrations of organic osmolytes.

Swelling-activated Organic Osmolyte Release Pathways in a Wide Range of Cell-Types have the Functional Characteristics of Channels

Directionality

The swelling-activated transport of organic osmolytes has been characterized in cells from a very wide range of organisms. In many cases exposure of cells to hypotonic media has been shown (using radiolabeled compounds) to cause an increase in the unidirectional rates of both efflux and influx for a range of solutes. The net flux of organic osmolytes is normally out of the cell. However, in investigating the detailed characteristics of swelling-activated transport pathways it is often convenient to measure unidirectional influx rates as this allows the experimenter full control over the solution from which the solute of interest approaches the membrane (i.e., the '*cis*' side). In such studies it is a common assumption that the pathway which mediates the increased unidirectional in-

flux of solute into swollen cells is the same as that responsible for swelling-activated efflux. This assumption has not routinely been tested, though in the few cases in which comparisons have been made between the functional (e.g., pharmacological) characteristics of swelling-activated efflux and influx pathways (e.g., for taurine) they have proven to be similar (e.g., Thoroed & Fugelli, 1994; Haynes & Goldstein, 1993; Hall, 1995; Hall et al., 1996). This is consistent with the pathways involved being bidirectional, with the direction of the net flux being determined by the prevailing electrochemical gradients and the law of mass-action.

Ion-Dependence

The swelling-activated transport of organic solutes in a wide range of cell-types has been found, in general, to be 'Na^+-independent'; i.e., swelling-activated influx is observed in cells bathed in media containing a range of different organic and inorganic cations in place of Na^+. In some cases the transport rates are influenced by the nature of the cation present; e.g., in C6 cells swelling-activated taurine and inositol influx is significantly reduced on replacement of Na^+ with *N*-methyl-D-glucamine (Jackson & Strange, 1993), whereas in human tracheal cells substitution of *N*-methyl-D-glucamine for Na^+ reportedly increases swelling-activated taurine efflux (Galietta et al., 1996). However, whether these effects are due to differential effects of the different cations on the solute translocation process, or to secondary effects arising from the effect of the cation-substitution on cell volume or some other parameter (e.g., intracellular pH) is not clear.

Replacement of Cl^- in the medium with some (though not all) other anions has been shown in some cases to alter swelling-activated transport rates for organic solutes; e.g., substitution of gluconate for Cl^- has been shown to reduce the rate of swelling-activated taurine and inositol efflux in a number of cell-types (Strange et al., 1993; Jackson & Strange, 1993; Galietta et al., 1996). Again, the basis for this is unclear, though it may well be a consequence of the high levels of the substituted anions used in these experiments simply blocking the efflux pathway and/or exerting their effect indirectly via their influence on the membrane potential.

Pharmacology

A variety of inhibitors of swelling-activated organic osmolyte transport have been identified (Table). In vertebrate cells, these include a range of 'traditional' anion transport inhibitors, unsaturated fatty acids, sulfhydryl reagents, metabolic inhibitors, and inhibitors of calmodulin and the cytochrome P450/lipoxygenase enzyme pathways. The pharmacological characteristics of swelling-activated transport vary somewhat between species and cell-types and it is, in most cases, unclear how such compounds actually exert their inhibitory effect. Nevertheless, such reagents have proven useful as a means of comparing the characteristics of swelling-activated transport of different solutes in individual cell-types. In a number of such studies the inhibitor-sensitivity of the swelling-activated transport of a range of different solutes has been compared and shown to be similar. Such data are consistent with the view that the swelling-activated transport of a range of structurally unrelated organic compounds is mediated by pathways of a single type (Kirk et al., 1992; Goldstein & Davis, 1994; Hall, 1995; Hall et al., 1996; Ruhfus & Kinne, 1996). As has been pointed out (Kinne et al., 1993; Napathorn & Spring, 1994; Hall et al., 1996) such pharmacological data are neither unambiguous nor conclusive. The existence of a number of different types of organic osmolyte permeation pathways, perhaps coexisting within single cell-types, cannot be ruled out. In cells from at least one tissue, the inner medullary collecting duct of the kidney, which is unusual in being exposed routinely to osmolalities much higher than that of the plasma, there is evidence for the existence of a sorbitol release mechanism that is distinct from a broad-specificity pathway which mediates the efflux of taurine and *myo*-inositol from the same cells (Ruhfus & Kinne, 1996). Nevertheless, for an increasingly wide range of cell-types, the hypothesis that the volume-regulatory efflux of a range of organic osmolytes (including sorbitol) is via common, broad-specificity pathways is the simplest explanation consistent with most of the available pharmacological data.

Kinetics, '*Trans*'-effects, Substrate Competition and Stereoselectivity

In those cases in which the kinetics of swelling-activated organic solute transport have been investigated, the influx rate has generally shown a linear dependence on solute concentration (e.g., Siebens & Spring, 1989; Kirk et al., 1992; Strange et al., 1993; Joyner & Kirk, 1994; Hall, 1995; Hall et al., 1996; Goldstein & Davis, 1994), up to concentrations of tens (and in some cases hundreds) of millimolar. High extracellular concentrations of solute have little effect on the swelling-activated efflux rate (e.g., Fincham et al., 1987; Haynes & Goldstein, 1993; Napathorn & Spring, 1994; Bursell et al., 1996); i.e., swelling-activated osmolyte efflux is not prone to '*trans*-stimulation.'

Attempts to demonstrate competition between different organic osmolytes by showing inhibition of the swelling-activated influx of one solute by higher concentrations of other solutes thought to permeate the same pathway have, with a few exceptions (e.g., Napathorn & Spring, 1994; Goldstein & Davis, 1994), proven unsuccessful.

Table. Inhibitors of swelling-activated organic osmolyte transport

Inhibitors	Cell-types
'Traditional' anion transport inhibitors	
4,4′Diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) 5-Nitro-2-(3-phenylpropylamino)-benzoate (NPPB) 2,5-Dichlorodiphenylamine-2-carboxylic acid (DCDPC) Niflumic acid Furosemide Dipyridamole Pyridoxal-5-phosphate	Fish erythrocytes (Wolowyk et al., 1989; Goldstein et al., 1991; Goldstein & Brill, 1991; Garcia Romeu et al., 1991; Motais et al., 1991, 1992; Kirk et al., 1992; Goldstein & Davis, 1994; Joyner & Kirk., 1994; Bursell & Kirk, 1996; Lewis et al., 1996) Skate hepatocytes (Ballatori et al., 1995) Rat glioma (C6) cells (Strange et al., 1993; Jackson & Strange, 1993) Rat astrocytes (Sánchez Olea et al., 1993; Gonzalez et al., 1995) Rat neurons (Sánchez Olea et al., 1996) Rabbit renal inner medullary collecting duct cells (Ruhfus & Kinne, 1996) Human erythroleukemic (K562) cells (Huang et al., 1996) Human cervical cancer (HeLa) cells (Hall et al., 1996) Human neuroblastoma cells (Basavappa et al., 1996)
Cationic channel blockers	
Quinine Quinidine La^{3+}	*Xenopus laevis* oocytes (Strange et al., 1996) Fish erythrocytes (Thoroed & Fugelli, 1994; Lewis et al., 1996) Dog kidney (MDCK) cells (Sánchez Olea et al., 1991) Rat astrocytes (Vitarella et al., 1994)
Unsaturated fatty acids	
Arachidonic acid Linolenic acid Linoleic acid	Rat glioma (C6) cells (Strange et al., 1993) Rat astrocytes (Sánchez Olea et al., 1995) Rat neurons (Sánchez Olea et al., 1996)
Sulfhydryl reagent	
N-Ethylmaleimide	Protozoa (Bursell et al., 1996) Fish erythrocytes (Bursell & Kirk, 1996) Skate hepatocytes (Ballatori et al., 1994)
Calmodulin antagonists	
Chlorpromazine Pimozide Trifluoperazine Tamoxifen and derivatives *N*-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide (W7)	Marine mollusc red blood cells (Pierce et al., 1989) Fish erythrocytes (Thoroed & Fugelli, 1994; Bursell & Kirk, 1996) Human cervical cancer (HeLa) cells (Kirk & Kirk, 1994) Rat cerebral cortex (Law, 1994a) Human neuroblastoma cells (Basavappa et al., 1996)
Lipoxygenase/Cytochrome P450 inhibitors	
Gossypol Ketoconazole Cinnamyl-3,4-dihydroxy-α-cyanocinnamate Eicosatetraenoic acid (ETYA) Nordihydroguaiaretic acid (NDGA)	Fish erythrocytes (Thoroed & Fugelli, 1994) Rabbit renal papillary (PAP-HT25) cells (Furlong et al., 1991) Rat glioma (C6) cells (Strange et al., 1993; Jackson & Strange, 1993) Ehrlich ascites tumour cells (Lambert & Hoffmann, 1993)
Metabolic inhibitors	
Azide 2,4-Dinitrophenol (DNP) Rotenone Carbonyl cyanide *p*-trifluoromethoxyphenyl-hydrazone (FCCP) 2-Deoxy-D-glucose	Skate hepatocytes (Ballatori et al., 1995) Rat glioma (C6) cells (Jackson et al., 1994)
Miscellaneous	
1,9-Dideoxyforskolin	Rat glioma (C6) cells (Strange et al., 1993; Jackson & Strange, 1993) Rat neurons (Sánchez Olea et al., 1996) Human lung cancer (S1) cells (Kirk & Kirk, 1993) Human cervical cancer (HeLa) cells (Hall et al., 1996)

The reagents are, for convenience, grouped under subheadings indicating the action for which each is, in most cases, best known. However, such compounds are not highly specific. All affect a range of cellular functions and the inclusion of a particular reagent under a particular heading is not necessarily indicative of its mode of action in inhibiting osmolyte transport. In particular, although compounds known to inhibit calmodulin and the cytochrome P450/lipoxygenase enzyme systems inhibit swelling-activated osmolyte transport in a range of cell-types, a role for these proteins/pathways in the regulation of swelling-activated transport remains, in most cases, unproven. The inhibitor-sensitivity of swelling-activated organic osmolyte transport does vary between cell types and not all the reagents listed under a particular subheading inhibit osmolyte transport in all of the cell-types listed. The studies cited are by way of example; the list is not exhaustive.

A number of studies have compared swelling-activated transport rates of stereoisomeric solutes (e.g., L- and D-alanine (Fincham et al., 1987); D- and L-glucose (Wolowyk et al., 1989); D- and L-sorbitol (Napathorn & Spring, 1994)) and have shown there to be little difference between them. The pathways involved are therefore not stereoselective.

In summary, the available data are consistent with the view that in many (though perhaps not all) cell-types the swelling-activated transport of a range of structurally unrelated organic solutes is via broad-specificity pathways of a single type. The apparent ability of these pathways to transport a range of structurally unrelated solutes in both directions across the cell membrane, their failure to saturate with increasing substrate concentrations, their inability to discriminate between stereoisomeric substrates, and their failure to show either *trans*-stimulation or (in most cases) substrate competition, indicate that their functional characteristics are unlike those of conventional transporters. Instead, they are those expected of a pore or channel.

There is Mounting Evidence that the Channels which Mediate Organic Osmolyte Release from Many Cell-Types are Anion Selective

Work over recent years has produced a number of independent lines of evidence implicating so-called 'volume-activated Cl^- channels' in the swelling-activated release of organic osmolytes. Data from electrophysiological studies suggest that there may well be a number of different types of anion-selective channel that are activated in response to cell swelling and which differ in their biophysical, pharmacological and regulatory characteristics (Fong & Jentsch, 1995; Strange et al., 1996). However, whole-cell electrical recordings of a very wide range of (vertebrate) cell-types exposed to hypo-osmotic media have revealed the presence of an outwardly-rectifying anion-selective channel and it is this channel that has been proposed to be the broad-specificity pathway involved in the swelling-activated efflux of both charged and uncharged organic solutes from vertebrate cells. In recognition of its proposed dual role in mediating the swelling-activated flux of inorganic anions and organic solutes, Strange and colleagues have termed this channel VSOAC, for Volume Sensitive organic Osmolyte and Anion Channel (Jackson et al., 1994).

Arguments in support of the hypothesis that anion-selective channels provide a major route for the volume-regulatory efflux of organic osmolytes from vertebrate cells are presented in some detail elsewhere (Strange & Jackson, 1995; Strange et al., 1996) and are summarized only briefly here:

(i) The swelling-activated transport of organic osmolytes is inhibited by a range of anion channel blockers. As discussed in the preceding section, such reagents are notoriously nonspecific and caution is necessary in analyzing and assessing experiments of this sort. Nevertheless, it has been shown in a number of cell-types that there is quantitative agreement between the effect of a range of structurally unrelated inhibitors on the swelling-activated transport of organic solutes and their effect on the swelling-activated transport of either Cl^- or I^- (both of which permeate swelling-activated anion channels) (Kirk & Kirk, 1993; Kirk & Kirk, 1994; Sánchez Olea et al., 1996; Lewis et al., 1996; Hall et al., 1996), as well as on swelling-activated whole-cell anion conductance (Jackson & Strange, 1993).

(ii) Swelling-activated organic osmolyte transport and swelling-activated anion channels show the same regulatory characteristics. Maneuvers that potentiate swelling-activated transport of taurine (e.g., exposure of cells to phorbol esters or transfection of cells with the human *mdr1* gene) have a very similar effect on the swelling-activated efflux of I^- (J.A. Hall, D.R. Gill, C.F. Higgins and K. Kirk, *unpublished*). Swelling-activated organic osmolyte transport (Jackson et al., 1994; Ballatori et al., 1995) and swelling-activation of whole-cell anion conductance (Gill et al., 1992; Oike et al., 1994; Jackson et al., 1994; Ballatori et al., 1995; Ruhfus & Kinne, 1996) show a similar dependence on the presence of cytosolic ATP. Furthermore, both are modulated in a similar way by the intracellular ionic composition (Strange et al., 1996), showing decreased activation with increasing intracellular ionic strength and/or Cl^- concentration.

(iii) Oocytes from the toad *Xenopus laevis* show a high degree of variability both in the magnitude of the swelling-activated anion conductance and the swelling-activated taurine efflux rate. However, there is a strong correlation between the two: i.e., oocytes with a large swelling-activated chloride conductance have a high rate of swelling-activated taurine efflux; those showing a low swelling-activated chloride conductance have a correspondingly low rate of taurine efflux (Hand et al., 1997). This is consistent with Cl^- and taurine sharing a common pathway.

(iv) Electrophysiological studies have demonstrated a significant permeability of swelling-activated anion channels to charged amino acids and other small organic solutes bearing a net negative charge (Banderali & Roy, 1992; Rasola et al., 1992; Jackson & Strange, 1993; Jackson et al., 1994; Roy, 1995; Basavappa et al., 1996; Boese et al., 1996). Such studies do not prove that such channels provide the major pathway for the volume-regulatory efflux of (predominantly neutral) organic osmolytes. However, they do constitute a clear demonstration that the permeability pathway provided by such channels is of sufficient dimensions to accommodate the compounds that serve as organic osmolytes.

(v) High concentrations of polyol osmolytes such as sorbitol and *myo*-inositol affect swelling-activated whole-cell chloride conductance in a way that is consistent with their competing with chloride for occupancy of the chloride channel (Jackson & Strange, 1993).

(vi) Several studies of the swelling-activated efflux of amino acids from vertebrate cells (e.g., Sanchez Olea, 1991; Roy & Malo, 1992) as well as protozoa (e.g., Bursell et al., 1996; Vieira et al., 1996) have shown there to be significant loss of neutral and anionic amino acids, but negligible loss of their similarly sized cationic counterparts, consistent with the pathway(s) involved being selective for anionic over cationic solutes.

The evidence that swelling-activated anion channels serve as the major route for the volume-regulatory efflux of organic osmolytes from many cell-types is persuasive, though not yet conclusive. It is largely correlative. Furthermore, it comes from relatively few studies and it is not at all clear that the mechanism of swelling-activated organic osmolyte efflux is the same in all vertebrate cells, let alone in cells of invertebrate species. A number of studies of different vertebrate cell-types have presented data consistent with separate routes for the swelling-activated efflux of taurine and Cl^-. For example, exposure of rat mammary gland to hypotonic media causes a marked increase in taurine efflux but has no effect on I^- efflux, from which it was concluded that taurine exits this tissue via a mechanism other than anion-channels (Shennan et al., 1994). Similarly (though in marked contrast to data obtained with eel erythrocytes; Lewis et al., 1996), skate erythrocytes in hypotonic media show activation of taurine efflux without there being a corresponding increase in the rate of Cl^- efflux (Davis-Amaral et al., 1996). The data from these studies have been taken to indicate the existence of taurine efflux pathways (channels ?) having a comparatively low Cl^- permeability. However, the study of Cl^- efflux from skate erythrocytes was carried out under conditions in which the efflux of Cl^- (but not taurine) was likely to have been rate-limited by the efflux of K^+ from the cells, rather than by the Cl^- permeability of the taurine release pathway; the permeability of the pathway to Cl^- was therefore not tested directly. Furthermore, in both of these studies it is not clear what pathways mediated the efflux of Cl^-/I^- in cells at their normal volume and how these pathways responded to hypotonic shock. Vertebrate cells have a variety of (constitutively active) exchangers, cotransporters and channels that mediate Cl^- transport and it is possible that alterations in the activity of these following cell swelling might mask the flux of Cl^- or I^- via swelling-activated anion channels.

A recent study with a human biliary cell line found the opposite results: osmotic swelling was followed by a marked increase in I^- efflux and by activation of an outwardly rectifying anion current; however, taurine efflux was reportedly unaffected (Roman et al., 1996). In Ehrlich ascites tumor cells osmotic swelling activates the efflux of both taurine and Cl^- (Hoffmann & Hendil, 1976; Lambert & Hoffman, 1993; Lambert & Hoffman, 1994). However, swelling-activated taurine efflux is potentiated by arachidonic and oleic acid but inhibited by DIDS, whereas swelling-activated Cl^- efflux is inhibited by the former and largely unaffected by the latter (Lambert & Hoffmann, 1994). Data such as these may reflect the existence of different types of swelling-activated, anion-selective channel, having different relative permeabilities to Cl^- and taurine and somewhat different pharmacological properties.

The Selectivity Properties of Organic Osmolyte Channels are Not Well Understood

Anions

Although the available evidence is consistent with the view that in many vertebrate cell-types swelling-activated organic osmolyte transport is via channels having a marked preference for anions and nonelectrolytes over cations, the detailed selectivity characteristics of these channels and of other putative osmolyte release pathways remain to be established.

Electrophysiological (reversal potential) measurements of the selectivity of the swelling-activated, outwardly-rectifying anion conductance observed in a wide range of cell-types have generally focused on inorganic anions, yielding in most cases (though with some slight variation between studies) the selectivity series $SCN^- > I^- > NO_3^- > Br^- > Cl^- > F^-$ (i.e., Eisenman series I). Such studies commonly include gluconate, a 6-carbon monovalent anionic polyol. This compound is usually found to have a permeability coefficient in the range of 0.05–0.35 times that of Cl^- (e.g. Díaz et al., 1993; Nilius et al., 1994; Chan et al., 1994; Verdon et al., 1995; Jackson et al., 1996), though $P_{gluconate}/P_{Cl}$ values as high as 0.70 have been reported (Voets et al., 1996).

In those studies in which the permeability of swelling-activated channels to organic anions (e.g., acetate, pyruvate, proprionate) has been investigated in more detail, small monovalent carboxylate anions have been found to have permeability coefficients typically in the range 0.05–0.40 that of Cl^- (e.g., Jackson et al., 1994). The permeability coefficients of divalent organic anions (e.g., malate, fumarate) are somewhat lower, with values usually $\leqslant 0.05$ that of Cl^- (Stoddard et al., 1993; Jackson et al., 1994; Roy, 1995), though in one study comparing swelling-activated anion conductances in a number of human cell lines the divalent sulfate ion was reported to have a permeability coefficient 0.12 times that of Cl^- (Rasola et al., 1992).

Amino Acids

There has been a number of electrophysiological studies of the permeability of swelling-activated channels to amino acids. Reversal potential measurements with the anionic amino acids aspartate and glutamate have yielded permeability coefficients relative to that of Cl^- within the range 0.08–0.20, with little difference between the two (Rasola et al., 1992; Banderali & Roy, 1992; Jackson et al., 1994; Chan et al., 1994; Roy, 1995). In the case of taurine, whole-cell experiments carried out at pH $\geqslant$ 8.2 and using very high concentrations of taurine (the purpose of both maneuvers being to ensure a sufficient concentration of the negatively charged form of the amino acid to produce measurable currents) indicate that the permeability of the channel in rat (C6) and human (U-138MG) glial cells to anionic taurine is 0.15–0.25 that to Cl^- (Jackson & Strange, 1993; Roy, 1995). Single channel reversal potential measurements on a kidney epithelial cell line (MDCK) indicate a somewhat higher taurine permeability, with $P_{taurine}/P_{Cl} = 0.49$ (Banderali & Roy, 1992).

Electrophysiological experiments such as these provide no information about the relative permeability of the channel to the neutral, zwitterionic form of taurine (which comprises >95% of the cytosolic taurine pool under physiological conditions). However, in eel erythrocytes, direct measurement of the relative rates of swelling-activated transport of $^{36}Cl^-$ and [^{14}C]taurine (under conditions in which the amino acid is present predominantly as an electroneutral zwitterion) yielded an apparent $P_{taurine}/P_{Cl}$ value of 0.26. This value is similar to the values obtained from electrophysiological (reversal potential) measurements on mammalian cells, and although channel selectivity does appear to vary significantly between species and cell-types this is at least consistent with the neutral form of the amino acid permeating the channel at a rate similar to the anionic form.

Estimates of the relative permeability of swelling-activated osmolyte channels to other amino acids, made using a number of different techniques, indicate that the permeability varies with the chemical structure of the amino acids. Measurements of the effect of extracellular amino acids on RVD in cultured astrocytes (Pasantes Morales et al., 1994) as well as direct electrophysiological measurements on cultured human glial cells (Roy, 1995) suggest that swelling-activated channels have a higher permeability to β-amino acids than to similarly sized α-amino acids. In several studies glutamate and apartate have been shown to have a permeability somewhat lower than that of similarly sized electroneutral amino acids (Pasantes Morales & Schousboe, 1988; Roy, 1995; Bursell et al., 1996), though in at least one study (Kimelberg et al., 1990) the rate of efflux of taurine, L-glutamate and D-aspartate from swollen cells has been shown to be similar.

Polyols

Estimates of the relative permeability of swelling-activated channels to electroneutral solutes come, in most cases, from flux studies. Experiments on a number of different cell-types have consistently shown the rate of permeation of taurine (i.e., the combined anionic and electroneutral forms) via swelling-activated pathways to be higher than that of a number of 6-carbon polyols including glucose, sorbitol and *myo*-inositol (Wolowyk et al., 1989; Kirk et al., 1992; Goldstein & Davis, 1994; Hall, 1995; Hall et al., 1996). Comparisons of the relative rates of transport of the latter two compounds in osmotically swollen cells have shown the permeability of sorbitol to be approximately twice that of *myo*-inositol (Siebens & Spring, 1989; Jackson & Strange, 1993; Hall, 1995) and some 4–6 times that of the disaccharide sucrose which, despite its relatively large size, does show significant swelling-activated transport (Siebens & Spring, 1989; Hall et al., 1996). Spring and colleagues have analyzed the rates of permeation of these and a range of other polyols in cultured rabbit kidney (papillary) cells (Siebens & Spring, 1989; Napathorn & Spring, 1994) in some detail. The data indicate that the rate of permeation is influenced by a combination of factors including solute size and the presence and arrangement of hydroxyl groups on the solute.

Nucleosides

The swelling-activated transport of pyrimidine nucleosides has been demonstrated in a number of different cell-types. These compounds, consisting of a 5-carbon sugar, ribose, linked to a hydrophobic pyrimidine base (i.e., a 6-membered ring), are relatively large. However, they have been shown in a number of studies to permeate swelling-activated pathways at a rate similar to or greater than substantially smaller polyols (Kirk et al., 1992; Hall, 1995; Hall et al., 1996). These data may be indicative of the permeation pathway being somewhat hydrophobic in character and having a preference for hydrophobic over hydrophilic solutes.

There have been a number of estimates of the minimum pore diameter for swelling-activated osmolyte/anion channels made on the basis of the size of solutes that either permeate or are excluded from the channel. Studies of the permeation of amino acids and 6-carbon polyols have yielded estimates in the range 5.4–5.9 Å. However the data from a number of different vertebrate cell-types, consistent with the view that pyrimidine nucleosides (with a minimum cylindrical diameter of ~8 Å) and perhaps, albeit at a much slower rate, the disaccharide, sucrose (with a minimum cylindrical diameter of ~9 Å) permeate channels of this sort suggest a somewhat

larger minimum pore diameter, in the range 8–9 Å (Hall et al., 1996).

Cations

There is conflicting data regarding the cation permeability of swelling-activated anion/osmolyte channels. Electrophysiological studies of swelling-activated anion conductance pathways suggest, in most cases, that such pathways have a low cation permeability (Strange et al., 1996). For the channel in rat C6 glioma cells the Cs^+ permeability was estimated as being only 0.04 times the Cl^- permeability (Jackson & Strange, 1993). Similarly, in three different human epithelial cell lines (P_{Na}/P_{Cl} was estimated as being in the range 0.02–0.05 (Rasola et al., 1992) and a recent study of human glial cells concluded that the swelling-activated anion conductance pathway had a negligible permeability to the small monovalent organic cation 3-amino-1-propanol (Roy, 1995). These data contrast with those from a number of other electrophysiological studies consistent with cell swelling activating an anion-selective channel with a more substantial permeability to both inorganic and organic cations (e.g., Chan et al., 1994; Verdon et al., 1995; Jackson et al., 1996). They also contrast with results from radiotracer flux studies carried out with a range of cell-types including erythrocytes from various fish species (Garcia-Romeu et al., 1991; Thoroed & Fugelli, 1994; Bursell & Kirk, 1995, 1996), chondrocytes isolated from bovine cartilage (Hall, 1995), and human cancer (HeLa cells) cells (Hall et al., 1996). These studies have revealed significant swelling-activated transport of both inorganic and organic cations ($^{86}Rb^+$ and choline respectively) via a pathway that is pharmacologically indistinguishable from that which mediates the swelling-activated transport of taurine.

It is quite likely that the cation permeability of swelling-activated anion/osmolyte channels varies between cell-types. Nevertheless, the postulated Rb^+ and choline permeability of the pathway in HeLa cells in particular (Hall et al., 1996) contrasts with the conclusion drawn from an earlier electrophysiological study on the same cell-type (Díaz et al., 1993). Whole-cell recordings made with an *N*-methyl-D-glucamine Cl^--rich solution in the pipette and a NaCl solution in the bath gave a reversal potential close to 0 mV. This was taken as evidence against the swelling-activated channel having a significant cation permeability.

The apparent discrepancy between the electrophysiological and flux data might be reconciled if the permeation of these channels by cations involves an interaction between the cation and permeant anions, with a significant proportion of the cation flux occurring in an 'electrically silent' manner, via the passage of electroneutral cation-anion pairs. In a recent study of the characteristics of swelling-activated $^{86}Rb^+$ transport in eel erythrocytes it was found that the rate of Rb^+ transport via a pathway pharmacologically indistinguishable from the taurine channel in these cells varied with the nature of the anion in the suspending medium (in the order $SCN^- > NO_3^- > CH_3SO_3^-$; J.D.H. Bursell, R.A. Lewis and K. Kirk, *unpublished*). This is as might be expected if a significant proportion of the Rb^+ flux was in the form of Rb^+-anion pairs, though other explanations cannot be excluded. The estimated minimum pore size for the organic osmolyte channel (8–9 Å) is certainly sufficient to accommodate such pairs of ions (e.g., the combined diameters of the Rb^+ ion (2.9 Å) and Cl^- ion (3.6 Å) is only 6.5 Å). Nevertheless the model remains largely speculative.

The hypothesis that swelling-activated osmolyte channels, despite being anion-selective, have a significant cation permeability raises the possibility that such channels make a significant contribution to the volume-regulatory efflux of K^+ from some cell-types. For such channels to mediate a net efflux of inorganic ions they would have to have a degree of selectivity for K^+ over Na^+. Whether or not this is the case has yet to be determined.

The Molecular Identity of these Channels Remains an Open and Controversial Question

The question of the molecular identity of organic osmolyte channels is a vexed one. As has been discussed, in some if not all vertebrate cells there is good reason to believe that this question is synonymous with that of the identity of the outwardly-rectifying, swelling-activated chloride channel. However the existence of distinct channels with a preference for organic over inorganic solutes cannot be ruled out and a recent study with a low molecular weight peptide ('phospholemman') provides some intriguing evidence for the existence of such proteins (*see* (v) below).

Within the last five years no fewer than six proteins have been postulated to have some association with swelling-activated anion-conductance and/or organic osmolyte transport. The proteins include:

(i) *ClC-2.* This protein is a member of a family of membrane proteins, several of which have been demonstrated to function as Cl^- channels (Brandt & Jentsch, 1995). There is good evidence that ClC-2 is itself a volume-sensitive, anion-selective channel (Gründer et al., 1992). However its selectivity, pharmacological and electrophysiological characteristics (at least when expressed in *Xenopus* oocytes) are quite different from those of the seemingly ubiquitous, swelling-activated, outwardly-rectifying anion channel.

(ii) *P-glycoprotein.* This protein is the product of the *mdr1* gene and is a member of the ABC transporter

family. Expression of P-glycoprotein is upregulated in drug-resistant cancer cells in which it acts to pump cytotoxic drugs from the cell cytosol. In addition to its drug-pumping function it has been postulated to serve as a swelling-activated anion channel and/or channel regulator. Cells transfected with *mdr1* have, in a number of studies, been shown to have a swelling-activated anion conductance that differs in magnitude and/or in its regulatory characteristics from that of non-transfected cells (Valverde et al., 1992; Gill et al., 1992; Hardy et al., 1995; Valverde et al., 1996). However, there is, in general, no correlation between levels of expression of P-glycoprotein and swelling-activated channel activity (McEwan et al., 1992; Rasola et al., 1994; Wang et al., 1994; Dong et al., 1994; Kunzelmann et al., 1994; De Greef et al., 1995*a,b*; Morin et al., 1995; Tominaga et al., 1995; Viana et al., 1995). Furthermore, although some compounds that inhibit P-glycoprotein-mediated drug pumping also inhibit swelling-activated anion channels (and osmolyte efflux) it is unlikely that they do so via an effect on P-glycoprotein (Kirk & Kirk, 1994). A physiological role for P-glycoprotein in the volume regulatory response therefore remains unproven.

(iii) *pI_{Cln}*. This 235 amino acid protein was cloned originally from MDCK cells (Paulmichl et al., 1992). When expressed in *Xenopus* oocytes it generates a Cl^- conductance that is constitutively active and which has characteristics similar to those of the swelling-activated anion/osmolyte channel (summarized by Strange et al., 1996). Monoclonal antibodies recognizing pI_{Cln} block activation of a native hypotonically induced Cl^- conductance in *Xenopus* oocytes (Krapivinsky et al., 1994). Furthermore, treatment of fibroblasts with antisense oligodeoxynucleotides to decrease the level of expression of this protein suppresses activation of the swelling-induced chloride current (Gschwentner et al., 1995), consistent with a role for this protein in swelling-activated channel activity in non-transfected cells.

Paulmichl et al. (1992) postulated that this protein forms a novel (dimeric) channel. However the subsequent finding by Krapivinsky et al. (1994) that the protein is located primarily in the cell cytoplasm led to the counter proposal that it serves as a cytosolic regulator of an endogenous, swelling-activated channel (Ackerman et al., 1994). This hypothesis is difficult to reconcile with the original observation of Paulmichl et al. (1992) that mutations in the protein alter the sensitivity of the channel to inhibition by extracellular nucleotides as well as the kinetics and Ca^{2+}-dependence of the channel. Paulmichl and colleagues have described preliminary results indicating that although the protein is normally present in the cytosol it migrates to the membrane in response to osmotic swelling (Paulmichl et al., 1996) and Strange et al. (1996) have proposed an 'anchor-insertion model' that might account for much of the reported data.

However, two recent studies by Nilius, Eggermont and colleagues cast significant doubts on the involvement of pI_{Cln} in swelling-activated channel activity. In the first of the two studies it was shown that the current induced in *Xenopus* oocytes by the expression of human pI_{Cln} differs from the endogenous swelling-activated current in its electrophysiological, pharmacological and biological characteristics (Voets et al., 1996). These data argue against the hypothesis that human pI_{Cln} activates (regulates) the native swelling-activated channel of *Xenopus* oocytes. They might be explained by human pI_{Cln} being a channel with characteristics somewhat different from those of its amphibian counterpart. However in a second paper from the same group it was shown that a current with the same characteristics as that induced in *Xenopus* oocytes by human pI_{Cln} can also be detected in a significant fraction of non-injected oocytes, as well as in oocytes expressing another unrelated human protein, ClC-6 (Buyse et al., 1997). These data are consistent with the view that the pI_{Cln}-induced current is mediated by a channel native to the oocyte, that this channel is distinct from the endogenous swelling-activated channel, that the channel is usually (though not always) inactive in non-injected oocytes, and that the activity of the channel is increased in response to the expression of foreign proteins. In the same paper it was also shown that a second conductance endogenous to *Xenopus* oocytes has the same characteristics as the current attributed by Paulmichl et al. (1992) to mutated forms of pI_{Cln}. Finally, it was shown that in endothelial cells pI_{Cln} is present predominantly in the cell cytosol, with no alteration in its intracellular distribution in response to osmotic swelling. These data argue against pI_{Cln} being either the swelling-activated channel or a regulator thereof.

(iv) *Band 3.* This protein is a major constituent of the plasma membrane of erythrocytes from most vertebrate species and has homologues in other tissues. Its normal mode of operation is as an electroneutral anion (Cl^-/HCO_3^-) exchange system. However the observation that many inhibitors of band 3-mediated anion exchange also inhibit swelling-activated anion/osmolyte channels has prompted the suggestion that band 3 (and its homologues in other tissues) might be involved in swelling-activated channel activity (Goldstein et al., 1990; Goldstein & Brill, 1991; Garcia Romeu et al., 1991; Motais et al., 1991, 1992). Expression of band 3 from trout erythrocytes (which have a swelling-activated osmolyte channel) in *Xenopus* oocytes results in increased anion-conductance and taurine permeability (Fiévet et al., 1995). By contrast, expression of band 3 from mouse erythrocytes (which lack swelling-activated osmolyte channel activity) does not. This is consistent with a role for the band 3 protein in the volume-regulatory response of fish erythrocytes (Garcia Romeu et al., 1996; Motais et al., 1997). However, the channel activity observed in

oocytes expressing trout band 3 is not volume-sensitive. Furthermore, it remains to be shown whether mammalian homologues of fish erythrocyte band 3 confer the same anion conductance/osmolyte permeability.

(v) *Phospholemman.* This is a small (72 amino acid) protein which when reconstituted into bilayers forms anion-selective channels that have an apparent permeability to the anionic form of taurine some 70 times higher than that to Cl^-, prompting the suggestion that it may be involved in volume-regulatory taurine transport (Moorman et al., 1995). The detailed functional characteristics of this channel and their degree of similarity with those of swelling-activated osmolyte channels remain to be established.

(vi) *VDAC.* The voltage-dependent anion channel (VDAC) is a 'porin-like' channel that is found in the outer membrane of eukaryotic mitochondria and which also has been postulated to be present in other membrane fractions including the plasma membrane (Reymann et al., 1995; Junankar et al., 1995). A note added in proof to a recent review by Reymann et al. (1995) referred to immunotopological evidence for the expression of VDAC in the plasma membrane of *Xenopus* oocytes and, furthermore, reported that antibodies against human (lymphocyte) VDAC inhibit hypotonically activated ion fluxes in these cells. These data are consistent with a role for VDAC in swelling-activated anion/osmolyte channel activity; however at this stage alternative explanations for the data cannot be ruled out.

In summary, despite a great deal of recent interest in the identity of the proteins involved in swelling-activated osmolyte/anion channel activity, the question remains an open one and for most of the candidate proteins the evidence linking them with the channels of wild-type cells is, at best, sparse.

Conclusion

The swelling-activated release of organic osmolytes plays a significant role in the volume-regulatory response of a very wide range of cell-types, ranging from protozoan parasites to mammalian cancer cells. The available data are consistent with the view that the transport pathways responsible for the volume-regulatory efflux of organic solutes are, in many cases and perhaps in general, broad-specificity channels. However, the existence in at least some cell-types of alternative pathways (channels) with a particular preference for certain organic solutes cannot at this stage be ruled out. There is compelling evidence from a range of vertebrate cell-types equating these broad-specificity channels with those giving rise to the swelling-activated outwardly-rectifying anion conductance that has been described in a very wide range of animal cells. However, the proof of this hypothesis awaits the unequivocal identification of the proteins involved and their functional expression and/or reconstitution into artificial membranes.

The broad specificity of the pathways involved in the volume-regulatory efflux of organic osmolytes has far-reaching implications for the cell. In addition to the relatively inert organic compounds that serve as the major organic osmolytes, cells contain at lower concentrations a wide variety of small organic compounds (biochemical intermediates, second messengers etc.) that are synthesized or accumulated by the cell at considerable expense and which serve a plethora of important functions. Activation of a channel with a significant permeability to such solutes will result in their wholesale loss from the cell (e.g., Hall et al., 1996), something not to be undertaken lightly. It is relevant to note that in the majority of studies of swelling-activated osmolyte transport and/or ion conductance the cells are subjected to a very substantial (grossly nonphysiological) hypotonic shock. In those relatively few studies in which the osmotic dependence of osmolyte channel activity has been characterized in detail it has been shown to be nonlinear: small increases in cell volume have little effect on channel activity; however, as the volume is increased further (beyond some threshold value) channel activity increases steeply (e.g., Kirk et al., 1992; Strange et al., 1993). Such data are consistent with the view that in vertebrate cells at least, swelling-activated osmolyte channels serve as an emergency system, to be activated as a means of dumping large amounts of solute quickly (albeit at considerable cost to the cell) under conditions in which the cells are in real danger of bursting. Whether these broad-specificity pathways with their ability to mediate the high capacity transport of a seemingly wide range of solutes might play other roles, either in the response of cells to the types of volume perturbation undergone in vivo, or perhaps in other aspects of cell function is the subject of ongoing research.

Work in this area in the author's laboratory at the University of Oxford was supported by the Lister Institute of Preventive Medicine, the Wellcome Trust, The Medical Research Council and The Royal Society. Work at the Australian National University was supported by the Australian National Health and Medical Research Council. I am grateful to the members of my lab, both past and present, who have contributed to this work, particularly James Bursell, James Hall, Ari Karjalainen, Julie Kirk, Rachel Lewis and Amanda Nourse. I am grateful also to Rolf Kinne, Kevin Strange and Stephen Wright for helpful comments made during the course of preparing this review.

References

Ackerman, M.J., Wickman, K.D., Clapham, D.E. 1994. Hypotonicity activates a native chloride current in *Xenopus* oocytes. *J. Gen. Physiol.* **103:**153–179

Altenberg, G.A., Deitmer, J.W., Glass, D.C., Reuss, L. 1994. P-Glycoprotein-associated Cl^- currents are activated by cell swelling but do not contribute to cell volume regulation. *Cancer Res.* **54:**618–622

Amende, L.M., Pierce, S.K. 1980. Cellular volume regulation in salinity stressed molluscs: the response of *Noetia ponderosa* (Arcidae)

red blood cells to osmotic variation. *J. Comp. Physiol.* **138:**283–289

Ballatori, N., Truong, A.T., Jackson, P.S., Strange, K., Boyer, J.L. 1995. ATP depletion and inactivation of an ATP-sensitive taurine channel by classic ion channel blockers. *Molecular Physiol.* **48:**472–476

Banderali, U., Roy, G. 1992. Anion channels for amino acids in MDCK cells. *Am. J. Physiol.* **263:**C1200–C1207

Basavappa, S., Huang, C.-C., Mangel, A.W., Lebedev, D.V., Knauf, P.A., Ellory, J.C. 1996. Swelling-activated amino acid efflux in the human neuroblastoma cell line CHP-100. *J. Neurophys.* **76:**764–769

Blum, J.J. 1992. Effect of osmolality on $^{86}Rb^+$ uptake and release by *Leishmania donovani*. *J. Cell. Physiol.* **152:**111–117

Boese, S.H., Wehner, F., Kinne, R.K.H. 1996. Taurine permeation through swelling-activated anion conductance in rat IMCD cells in primary culture. *Am. J. Physiol.* **40:**F498–F507

Boyd, T.A., Cha, C.J., Forster, R.P., Goldstein, L. 1977. Free amino acids in tissues of the little skate *Raja erinacea* and the stingray *Dasyatis sabina:* effects of environmental dilution. *J. Exp. Zool.* **199:**435–442

Brand, H.S., Meijer, A.J., Gustafson, L.A., Jörning, G.G.A., Leegwater, A.C.J., Maas, M.A.W., Chamuleau, R.A.F.M. 1994. Cell-swelling-induced taurine release from isolated perfused rat liver. *Biochem. Cell Biol.* **72:**8–11

Brandt, S., Jentsch, T.J. 1995. ClC-6 and ClC-7 are two novel broadly expressed members of the CLC chloride channel family. *FEBS Lett.* **377:**15–20

Brill, S.R., Musch, M.W., Goldstein, L. 1992. Taurine efflux, band 3, and erythrocyte volume of the hagfish (*Myxine glutinosa*) and lamprey (*Petromyzon marinus*). *J. Exp. Zool.* **264:**19–25

Burg, M.B. 1994. Molecular basis for osmoregulation of organic osmolytes in renal medullary cells. *J. Exp. Biol.* **268:**171–175

Bursell, J.D.H., Kirk, K. 1995. Two functionally distinct swelling-activated K^+ transport pathways in eel erythrocytes. *J. Physiol.* **433:**P181–P182

Bursell, J.D.H., Kirk, K. 1996. Swelling-activated K^+ transport via two functionally distinct pathways in eel erythrocytes. *Am. J. Physiol.* **270:**R61–R70

Bursell, J.D.H., Kirk, J., Hall, S.T., Gero, A.M., Kirk, K. 1996. Volume-regulatory amino acid release from the protozoan parasite *Crithidia luciliae*. *J. Membrane Biol.* **154:**131–141

Buyse, G., Voets, T., Tytgat, J., De Greef, C., Droogmans, G., Nilius, B., Eggermont, J. 1997. Expression of human pI_{Cln} and ClC-6 in *Xenopus* oocytes induces an identical endogenous chloride conductance. *J. Biol. Chem.* **272:**3615–3621

Chan, H.C., Fu, W.O., Chung, Y.W., Huang, S.J., Chan, P.S.F., Wong, P.Y.D. 1994. Swelling-induced anion and cation conductances in human epididymal cells. *J. Physiol.* **478.3:**449–460

Darling, T.N., Burrows, C.M., Blum, J.J. 1990. Rapid shape change and release of ninhydrin-positive substances by *Leishmania major* promastigotes in response to hypo-osmotic stress. *J. Protozool.* **37:**493–499

Davis-Amaral, E., Musch, M.W., Goldstein, L. 1996. Chloride and taurine effluxes occur by different pathways in skate erythrocytes. *Am. J. Physiol.* **271:**R1544–R1549

Deaton, L.E. 1994. Hypo-osmotic volume regulation in bivalves: protein kinase C and amino acid release. *J. Exp. Zool.* **268:**145–150

De Greef, C., Sehrer, J., Viana, F., van Acker, K., Eggermont, J., Mertens, L., Raeymaekers, L., Droogmans, G., Nilius, B. 1995a. Volume-activated chloride currents are not correlated with P-glycoprotein expression. *Biochem. J.* **307:**713–718

De Greef, C., van der Heyden, S., Viana, F., Eggermont, J., De Bruijn, E.A., Raeymaekers, L., Droogmans, G., Nilius, B. 1995b. Lack of correlation between mdr-1 expression and volume-activation of chloride currents in rat colon cancer cells. *Pfluegers Arch.* **430:**296–298

Deutsch, C., Lee, S.C. 1988. Cell volume regulation in lymphocytes. *Renal Physiol. Biochem.* **11:**260–276

Díaz, M., Valverde, M.A., Higgins, C.F., Rucareanu, C., Sepúlveda, F.V. 1993. Volume-activated chloride channels in HeLa cells are blocked by verapamil and dideoxyforskolin. *Pfluegers Arch.* **422:**347–353

Dickman, K.G., Goldstein, L. 1990. Cell volume regulation by skate erythrocytes: role of potassium. *Am. J. Physiol.* **258:**R1217–R1223

Dong, Y., Chen, G., Duran, G.E., Kouyama, K., Chao, A.C., Sikic, B.I., Gollapudi, S.V., Gupta, S., Gardner, P. 1994. Volume-activated chloride current is not related to P-glycoprotein overexpression. *Cancer Res.* **54:**5029–5032

Fiévet, B., Gabillat, N., Borgese, F., Motais, R. 1995. Expression of band 3 anion exchange induces chloride current and taurine transport: structure-function analysis. *EMBO J.* **14:**5158–5169

Fincham, D.A., Wolowyk, M.W., Young, J.D. 1987. Volume-sensitive taurine transport in fish erythrocytes. *J. Membrane Biol.* **96:**45–56

Fincham, D.A., Wolowyk, M.W., Young, J.D. 1990. Characterization of amino acid transport in red blood cells of a primitive vertebrate, the Pacific Hagfish (*Ptatretus stouti*). *J. Exp. Biol.* **154:**355–370

Fong, P., Jentsch, T.J. 1995. Molecular basis of epithelial Cl channels. *J. Membrane Biol.* **144:**189–197

Fugelli, K., Thoroed, S.M. 1986. Taurine transport associated with cell volume regulation in flounder erythrocytes under anisosmotic conditions. *J. Physiol.* **374:**245–261

Furlong, T.J., Moriyama, T., Spring, K.R. 1991. Activation of osmolyte efflux from cultured renal papillary epithelial cells. *J. Membrane Biol.* **123:**269–277

Galietta, L.V.J., Romeo, G., Zegarramoran, O. 1996. Volume-regulatory taurine release in human tracheal 9HTEO(−) and multidrug resistant 9HTEO(−)/DX cells. *Am. J. Physiol.* **267:**C728–C735

Garcia-Romeu, F., Cossins, A.R., Motais, R. 1991. Cell volume regulation by trout erythrocytes: characteristics of the transport systems activated by hypotonic swelling. *J. Physiol.* **440:**547–567

Garcia-Romeu, F., Borgese, F., Guizouarn, H., Fievet, B. Motais, R. 1996. A role for the anion exchanger AE1 (Band 3 protein) in cell volume regulation. *Cell Molec. Biol.* **42:**985–994

Geoffrion, Y., Larochelle, J. 1984. The free amino acid contribution to osmotic regulation in *Acanthamoeba castellani*. *Can. J. Zool.* **62:**1954–1959

Gill, D.R., Hyde, S.C., Higgins, C.F., Valverde, M.A., Mintenig, G.M., Sepulveda, F.V. 1992. Separation of drug transport and chloride channel functions of the human multidrug resistance P-glycoprotein. *Cell* **71:**23–32

Goldstein, L., Brill, S.R., Freund, E.V. 1990. Activation of taurine efflux in hypotonically stressed elasmobranch cells: inhibition by stilbene disulfonates. *J. Exp. Zool.* **254:**114–118

Goldstein, L., Brill, S.R. 1991. Volume-activated taurine efflux from skate erythrocytes: possible band 3 involvement. *Am. J. Physiol.* **260:**R1014–R1020

Goldstein, L., Davis, E.M. 1994. Taurine, betaine and inositol share a volume-sensitive transporter in skate erythrocyte cell membrane. *Am. J. Physiol.* **267:**R426–R431

Gonzalez, E., Sánchez Olea, R., Pasantes Morales, H. 1995. Inhibition by Cl^- channel blockers of the volume-activated diffusional mechanism of inositol transport in primary astrocytes in cultures. *Neurochem. Res.* **20:**895–900

Gründer, S., Thiemann, A., Pusch, M., Jentsch, T.J. 1992. Regions involved in the opening of ClC-2 chloride channel by voltage and cell volume. *Nature* **360:**759–763

Gschwentner, M., Nagl, U.O., Woll, E., Schmarda, A., Riter, M., Paulmichl, M. 1995. Antisense oligonucleotides suppress cell-volume-induced activation of chloride channels. *Pfluegers Arch.* **430:**464–470

Hall, A.C. 1995. Volume-sensitive taurine transport in bovine articular chondrocytes. *J. Physiol.* **484:**755–766

Hall, J.A., Kirk, J., Potts, J.R., Rae, C., Kirk, K. 1996. Anion channel blockers inhibit swelling-activated anion, cation, and nonelectrolyte transport in HeLa cells. *Am. J. Physiol.* **271:**C579–C588

Hallows, K.R., Knauf, P.A. 1994. Regulatory volume decrease in HL-60 cells: importance of rapid changes in permeability of Cl^- and organic solutes. *Am. J. Physiol.* **267:**C1045–C1056

Hand, M., Morrison, R., Strange, K. 1997. Characterization of volume-sensitive organic osmolyte efflux and anion current in *Xenopus* oocytes. *J. Membrane Biol.* **157:**9–16

Hardy, S.P., Goodfellow, H.R., Valverde, M.A., Gill, D.R., Sepulveda, F.V., Higgins, C.F. 1995. Protein kinase C-mediated phosphorylation of the human multidrug resistance P-glycoprotein regulates cell volume-activated chloride channels. *EMBO J.* **14:**68–75

Haynes, J.K., Goldstein, L. 1993. Volume-regulatory amino acid transport in erythrocytes of the little skate, *Raja erinacea*. *Am. J. Physiol.* **265:**R173–R179

Hoffmann, E.K., Hendil, K.B. 1976. The role of amino acids and taurine in isosmotic intracellular regulation in Ehrlich ascites mouse tumour cells. *J. Comp. Physiol.* **108:**279–286

Hoffmann, E.K., Simonsen, L.O., Lambert, I.H. 1984. Volume-induced increase of K^+ and Cl^- permeabilities in Ehrlich ascites tumour cells. Role of internal Ca^{2+}. *J. Membrane Biol.* **78:**211–222

Huang, C.-C., Basavappa, S., Ellory, J.C. 1996. Volume-activated taurine permeability in cells of the human erythroleukemic cell line K562. *J. Cell Physiol.* **167:**354–358

Huxtable, R.J. 1992. Physiological actions of taurine. *Physiological Rev.* **72:**101–163

Jackson, P.S., Strange, K. 1993. Volume-sensitive anion channels mediate swelling-activated inositol and taurine efflux. *Am. J. Physiol.* **265:**C1489–C1500

Jackson, P.S., Morrison, R. Strange, K. 1994. The volume-sensitive organic osmolyte-anion channel VSOAC is regulated by nonhydrolytic ATP binding. *Am. J. Physiol.* **267:**C1203–C1209

Jackson, P.S., Strange, K. 1995. Single-channel properties of a volume-sensitive anion conductance. Current activation occurs by abrupt switching of closed channels to an open state. *J. Gen. Physiol.* **105:**643–660

Jackson, P.S., Churchwell, K., Ballatori, N., Boyer J.L., Strange, K. 1996. Swelling-activated anion conductance in skate hepatocytes: regulation by cell Cl^- and ATP. *Am. J. Physiol.* **270:**C57–C66

Jensen, F.B. 1995. Regulatory volume decrease in carp red blood cells: mechanisms and oxygenation-dependency of volume-activated potassium and amino acid transport. *J. Exp. Biol.* **198:**155–165

Joyner, S.E., Kirk, K. 1994. Two pathways for choline transport in eel erythrocytes: a saturable carrier and a volume-activated channel. *Am. J. Physiol.* **267:**R773–R779

Junankar, P.R., Dulhunty, A.F., Curtis, S.M., Pace, S.M., Thinnes, F.P. 1995. Porin-type1 proteins in sarcoplasmic reticulum and plasmalemma of striated muscle fibres. *J. Muscle Research and Cell Motility* **16:**595–610

Kimelberg, H.K., Goderies, S.K., Higman, S., Pang, S., Waniewski, R.A. 1990. Swelling-induced release of glutamate, aspartate and taurine from astrocyte cultures. *J. Neurosci.* **10:**1583–1591

Kinne, R.K.H., Czekay, R.-P., Grunewald, J.M., Mooren, F.C., Kinne-Saffran, E. 1993. Hypotonicity-evoked release of organic osmolytes from distal renal cells: systems, signals, and sidedness. *Renal Physiol. Biochem.* **16:**66–78

Kirk, J., Kirk, K. 1994. Inhibition of volume-activated I^- and taurine efflux from HeLa cells by P-glycoprotein blockers correlates with calmodulin inhibtion. *J. Biol. Chem.* **269:**29389–29394

Kirk, K., Ellory, J.C., Young, J.D. 1992. Transport of organic substrates via a volume-activated channel. *J. Biol. Chem.* **267:**23475–23478

Kirk, K., Kirk, J. 1993. Volume-regulatory taurine release from a human lung cancer cell line: evidence for amino acid transport via a volume-activated chloride channel. *FEBS Lett.* **336:**153–158

Knodler, L.A., Edwards, M.R., Schofield, P.J. 1994. The intracellular amino acid pools of *Giardia intestinalis*, *Trichomonas vaginalis*, and *Crithidia luciliae*. *Exp. Parasitology* **79:**117–125

Krapivinsky, G.B., Ackerman, M.J., Gordon, E.A., Krapivinsky, L.D., Clapham, D.E. 1994. Molecular characterization of a swelling-induced chloride conductance regulatory protein, pI_{Cln}. *Cell* **76:**439–448

Kunzelmann, K., Slotki, I.N., Klein, P., Ausiello, D.A., Greger, R., Cabantchik, Z.I. 1994. Effects of P-glycoprotein expression on cyclic AMP and volume-activated ion fluxes and conductances in HT-29 colon adenocarcinoma cells. *J. Cell Physiol.* **161:**393–406

Lambert, I.H., Hoffmann, E.K. 1993. Regulation of taurine transport in Ehrlich ascites tumor cells. *J. Membrane Biol.* **131:**67–79

Lambert, I.H., Hoffman, E.K. 1994. Cell swelling activates separate taurine and chloride channels in Ehrlich mouse ascites tumor cells. *J. Membrane Biol.* **142:**289–298

Law, R.O. 1994a. Taurine efflux and the regulation of cell volume in incubated slices of rat cerebral cortex. *Biochim. Biophys. Acta* **1221:**21–28

Law, R.O. 1994b. Effects of extracellular bicarbonate ions and pH on volume-regulatory taurine efflux from rat cerebral cortical slices in vitro: evidence for separate neutral and anionic transport mechanisms. *Biochim. Biophys. Acta* **1224:**377–383

Lewis, R.A., Bursell, J.D.H., Kirk, K. 1996. Anion-selectivity of the swelling-activated osmolyte channel in eel erythrocytes. *J. Membrane Biol.* **149:**103–111

McEwan, G.T.A., Hunter, J., Hirst, B.H., Simmons, N.L. 1992. Volume-activated Cl^- secretion and transepithelial vinblastine secretion mediated by P-glycoprotein are not correlated in cultured human T84 intestinal epithelial layers. *FEBS Lett.* **304:**233–236

Moorman, J.R., Ackerman, S.J., Kowdley, G.C., Griffin, M.P., Mounsey, J.P., Chen, Z., Cala, S.E., O'Brian, J.J., Szabo, G., Jones, L.R. 1995. Unitary anion currents through phospholemman channel molecules. *Nature* **377:**737–740

Morán, J., Maar, T.E., Pasantes-Morales, H. 1994. Impaired volume regulation in taurine deficient cultured astrocytes. *Neurochem. Res.* **19:**415–420

Morin, X.K., Bond, T.D., Loo, T.W., Clarke, D.M., Bear, C.E. 1995. Failure of P-glycoprotein (MDR1) expressed in *Xenopus* oocytes to produce swelling-activated chloride channel activity. *J. Physiol.* **486:**707–714

Motais, R., Fiévet, B., Borgese, F., Garcia-Romeu, F. 1992. Some functional properties of band 3 protein in nucleated red cells. *Progr. Cell Res.* **2:**253–262

Motais, R., Guizouarn, H., Garcia-Romeu, F. 1991*b*. Red cell volume regulation: the pivotal role of ionic strength in controlling swelling-dependent transport systems. *Biochim. Biophys. Acta.* **1075:**169–180

Motais, R., Fiévet, B., Borgese, F., Garcia-Romeu, F. 1997. Association of the band 3 protein with a volume-activated anion and amino acid channel: a molecular approach. *J. Exp. Biol.* **200:**361–367

Mountian, I., Declerq, P.E., Van Driessche, W. 1996. Volume regulation in rat brain glial cells: lack of a substantial contribution of free amino acids. *Am. J. Physiol.* **270:**C1319–C1325

Nakanishi, T,. Balaban, R.S., Burg, M.B. 1988. Survey of osmolytes in renal cell lines. *Am. J. Physiol.* **255:**C181–C191

Napathorn, S., Spring, K. 1994. Further characterization of the sorbitol permease in PAP-HT25 cell. *Am. J. Physiol.* **267:**C514–C519

Neufeld, D.S., Wright, S.H. 1996. Salinity change and cell volume: the

response of tissues from the estuarine mussel *Geukensia demissa*. *J. Exp. Biol.* **199:**1619–1630

Neufeld, D.S., Wright, S.H. 1996. Response of cell volume in *Mytilus* gill to acute salinity change. *J. Exp. Biol.* **199:**473–484

Nikinmaa, M., Tufts, B.L., Boutilier, R.G. 1993. Volume and pH regulation in agnathan erythrocytes—comparisons between the hagfish, *Myxine glutinosa*, and the lampreys, *Petromyzon marinus* and *Lampetra fluviatilis*. *J. Comp. Physiol.* **163:**608–613

Nilius, B., Sehrer, J., Droogmans, G. 1994. Permeation properties and modulation of volume-activated Cl^- currents in human endothelial cells. *Br. J. Pharmacol.* **112:**1049–1056

Nonnotte, G., Truchot, J.-P. 1992. Cell volume regulation by erythrocytes of the euryhaline fish, *Platichthys flesus*, after hyposmotic stress in bicarbonate/carbon-dioxide-buffered medium. *Cell Physiol. Biochem.* **2:**336–348

Oike, M., Droogmans, G., Nilius, B. 1994. The volume-activated chloride current in human endothelial cells depends on intracellular ATP. *Pfluegers Arch.* **427:**184–186

Park, J.H., Schofield, P.J., Edwards, M.R. 1995. The role of alanine in the acute response of Giardia intestinalis to hypo-osmotic shock. *Microbiology* **141:**2455–2462

Pasantes Morales, H., Schousboe, A. 1988. Volume regulation in astrocytes: a role for taurine as an osmoeffector. *J. Neurosci. Res.* **20:**505–509

Pasantes Morales, H., Murray, R.A., Sánchez-Olea, R., Morán, J. 1994. Regulatory volume decrease in cultured astrocytes II. Permeability pathway to amino acids and polyols. *Am. J. Physiol.* **266:**C172–C178

Paulmichl, M., Li, Y., Wickman, K., Ackerman, M., Peralta, E., Clapham, D. 1992. New mammalian chloride channel identified by expression cloning. *Nature* **356:**238–241

Paulmichl, M., Laich, A., Fürst, H., Gschwentner, M., Nagl, U.O., Hittmair, A., Ritter, M. 1996. Transportation of the chloride channel I_{Cln} from the cytosol into the cell membrane after volume stress. *Biophys. J.* **70:**A9

Pierce, S.K., Greenberg, M.J. 1972. The nature of cellular volume regulation in marine bivalves. *J. Exp. Biol.* **57:**681–692

Pierce, S.K., Greenberg, M.J. 1973. The initiation and control of free amino acid regulation of cell volume regulation in salinity stressed marine bivalves. *J. Exp. Biol.* **59:**435–440

Pierce, S.K., Politis, A.D., Cronkite, D.H., Rowland, L.M., Smith, L.H. 1989. Evidence of calmodulin involvement in cell volume recovery following hypo-osmotic stress. *Cell Calcium* **10:**159–169

Rasola, A., Galietta, L.J.V., Gruenert, D.C., Romeo, G. 1992. Ionic selectivity of volume-sensitive currents in human epithelial cells. *Biochim. Biophys. Acta* **1139:**319–323

Rasola, A., Galietta, L.J., Gruenert, D.C., Romeo, G. 1994. Volume-sensitive chloride currents in four epithelial cell lines are not directly correlated to the expression of the MDR-1 gene. *J. Biol. Chem.* **269:**1432–1436

Reymann, S., Flörke, H., Heiden, M., Jakob, C., Stadtmüller, U., Steinacker, P., Lalk, V.E., Pardowitz, I., Thinnes, F.P. 1995. Further evidence for multitopological localization of mammalian porin (VDAC) in the plasmalemma forming part of a chloride channel complex affected in cystic fibrosis and encephalomyopathy. *Biochemical and Molecular Medicine* **54:**75–87

Roman, R.M., Wang, Y. Fitz, J.G. 1996. Regulation of cell volume in a human biliary cell line: activation of K^+ and Cl^- currents. *Am. J. Physiol.* **271:**G239–G248

Roy, G. 1995. Amino acid current through anion channels in cultured human glial cells. *J. Membrane Biol.* **147:**35–44

Roy, G., Sauvé, R. 1987. Effect of anisotonic media on volume, ion and amino acid content and membrane potential of kidney cells (MDCK) in culture. *J. Membrane Biol.* **100:**83–96

Roy, G., Malo, C. 1992. Activation of amino acid diffusion by a volume increase in cultured kidney (MDCK) cells. *J. Membrane Biol.* **130:**83–90

Ruhfus, B., Kinne, R.K.H. 1996. Hypotonicity-activated efflux of taurine and *myo*-inositol in rat inner medullary collecting duct cells: evidence for a major common pathway. *Kidney Blood Press. Res.* **19:**317–324

Sánchez Olea, R., Pasantes Morales, H., Lazaro, A., Cereijido, M. 1991. Osmolarity-sensitive release of free amino acids from cultured kidney cells (MDCK). *J. Membrane Biol.* **121:**1–9

Sánchez Olea, R., Pena, C., Morán, J., Pasantes Morales, H. 1993. Inhibition of volume regulation and efflux of osmoregulatory amino acids by blockers of Cl^- transport in cultured astrocytes. *Neurosci. Lett.* **156:**141–144

Sánchez Olea, R., Morales-Mulia, M., Morán, J., Pasantes Morales, H. 1995. Inhibition by polyunsaturated fatty acids of cell volume regulation and osmolyte fluxes in astrocytes. *Am. J. Physiol.* **269:**C96–C102

Sánchez Olea, R., Morales, M., Garcia, O., Pasantes Morales, H. 1996. Cl channel blockers inhibit the volume-activated efflux of Cl and taurine in cultured neurons. *Am. J. Physiol.* **39:**C1703–C1708

Shennan, D.B., McNeillie, S.A., Curran, D.E. 1993. Stimulation of taurine efflux from human placental tissue by a hyposmotic challenge. *Exp. Physiol.* **78:**843–846

Shennan, D.B., McNeillie, S.A., Curran, D.E. 1994. The effect of a hyposmotic shock on amino acid efflux from lactating rat mammary tissue: stimulation of taurine and glycine efflux via a pathway distinct from anion exchange and volume-activated anion channels. *Exp. Physiol.* **79:**797–808

Siebens, A.W., Spring, K.R. 1989. A novel sorbitol transport mechanism in cultured renal papillary epithelial cells. *Am. J. Physiol.* **257:**F937–F946

Smith, L.H., Pierce, S.K. 1987. Cell volume regulation by molluscan erythrocytes during hypoosmotic stress: Ca^{2+} effects on ionic and organic osmolyte effluxes. *Biol. Bull.* **173:**407–418

Stoddard, J.S., Steinbach, J.H., Simchowitz, L. 1993. Whole cell Cl^- currents in human neutrophils induced by cell swelling. *Am. J. Physiol.* **265:**C156–C165

Strange, K., Morrison, R. 1992. Volume regulation during recovery from chronic hypertonicity in brain glial cells. *Am. J. Physiol.* **263:**C412–C419

Strange, K., Jackson, P.S. 1995. Swelling-activated organic osmolyte efflux: a new role for anion channels. *Kidney Int.* **48:**994–1003

Strange, K., Emma, F., Jackson, P.S. 1996. Cellular and molecular physiology of volume-sensitive anion channels. *Am. J. Physiol.* **270:**C711–C730

Strange, K., Morrison, R., Shrode, L., Putnam, R. 1993. Mechanism and regulation of swelling-activated inositol efflux in brain glial cells. *Am. J. Physiol.* **265:**C244–C256

Thoroed, S.M., Fugelli, K. 1994. The Na^+-independent taurine influx in flounder erythrocytes and its association with the volume-regulatory taurine efflux. *J. Exp. Biol.* **186:**245–268

Tominaga, M., Tominaga, T., Miwa, A., Okada, Y. 1995. Volume-sensitive chloride channel activity does not depend on P-glycoprotein. *J. Biol. Chem.* **270:**27887–27893

Valverde, M.A., Díaz, M., Sepúlveda, F.V., Gill, D.R., Hyde, S.C., Higgins, C.F. 1992. Volume-regulated chloride channels associated with the human multidrug-resistance P-glycoprotein. *Nature* **355:**830–833

Valverde, M.A., Bond, T.D., Hardy, S.P., Taylor, J.C., Higgins, C.F., Altamirano, J., Alvarez-Leefmans, F.J. 1996. The multidrug resistance P-glycoprotein modulates cell regulatory volume decrease. *EMBO J.* **15:**4460–4468

Verdon, B., Winpenny, J.P., Whitfeld, K.J., Argent, B.E., Gray, M.A.

1995. Volume-activated chloride currents in pancreatic duct cells. *J. Membrane Biol.* **147:**173–183

Viana, F., Van Acker, K., De Greef, C., Eggermont, J., Raeymaekers, L., Droogmans, G., Nilius, B. 1995. Drug-transport and volume-activated chloride channel functions in human erythroleukemia cells: relation to expression level of P-glycoprotein. *J. Membrane Biol.* **145:**87–98

Vieira, L.L., Lafuente, E., Gamarro, F., Cabantchik, Z.I. 1996. An amino acid channel activated by hypotonically induced swelling of *Leishmania major* promastigotes. *Biochem. J.* **319:**691–697

Virkki, L.V., Nikinmaa, M. 1993. Regulatory volume decrease in lamprey erythrocytes: mechanisms of K^+ and Cl^- loss. *Am. J. Physiol.* **268:**R590–R597

Voets, T., Buyse, G., Tytgat, J., Droogmans, G., Eggermont, J., Nilius, B. 1996. The chloride current induced by expression of the protein pI_{Cln} in *Xenopus* oocytes differs from the endogenous volume-sensitive chloride current. *J. Physiol.* **495.2:**441–447

Wang, X., Wall, D.M., Parkin, J.D., Zalcberg, J.R., Kemm, R.E. 1994. P-glycoprotein expression in classical multi-drug resistant cells does not correlate with enhanced chloride channel activity. *Clin. Exp. Pharmacol. Physiol.* **21:**101–108

Wolowyk, M.W., Fincham, D.A., Young, J.D. 1989. The effects of furosemide, piretanide and MK-196 on volume sensitive solute transport in fish erythrocytes. *Proc. West. Pharmacol. Soc.* **32:**309–311

Yancey, P.H. 1994. Compatible and counteracting solutes. *In:* Cellular and Molecular Physiology of Cell Volume Regulation. (K. Strange, editor.) pp. 81–109. CRC Press, Boca Raton