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VOLUME REGULATION OF CHINESE HAMSTER OVARY CELLS IN ANISOOSMOTIC MEDIA

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Chinese hamster ovary (CHO) cells when suspended in anisoosmotic media regulate their volumes by the activation of specific ion transport pathways. In hypoosmotic media the cells first swell and then return to their isoosmotic volumes by the loss of cellular KCl and osmotically obliged water. This regulatory volume decrease (RVD) is insensitive to ouabain or bumetanide but is blocked by quinine, cetiedil and oligomycin C. Based on cell volume and membrane potential measurements under various experimental conditions, we conclude that hypoosmotic shock activates independent, conductive transport pathways for K^+ and for Cl^-, respectively. The anion pathway can also transport NO_3^- and SCN^- but not gluconate$^-$ anions. Osmotic shrinkage of CHO cells does not produce a regulatory volume increase (RVI) unless the cells have previously undergone a cycle of RVD. RVI is a Na^+-dependent, amiloride-sensitive, but ouabain- and oligomycin-insensitive process, probably involving a Na^+-H^+ exchange system. Internal acidification of isoosmotic cells by addition of a permeable weak acid also activates an amiloride-sensitive Na^+-H^+ exchange, producing a volume increase. Both RVD and RVI in CHO cells seem to involve molecular mechanisms similar to those described for the volume regulation of lymphocytes, indicating the prevalence of these phenomena in nucleated mammalian cells. Cultured CHO cell lines may provide a basis for a genetic characterization of the volume-regulatory transport pathways.

Introduction

Several types of cells when exposed to anisoosmotic media are reported to regulate their volumes: following an initial osmotic swelling or shrinking they return to their isoosmotic volumes. The types of cells that have been studied in most detail include nucleated red blood cells of birds, amphibians, and fish, epithelial cells of various origins, Ehrlich ascites cells and lymphoid cells (for reviews, see Refs. 1–6). Although different cell types appear to possess similar volume regulatory capacities, it is not clear that the underlying mechanisms are similar. For example, in lymphoblasts and peripheral blood or thymus lymphocytes the regulatory volume decrease (RVD) in hypoosmotic media has been shown to be produced by the opening of independent, conductive K^+ and Cl^- pathways [6–13], while regulatory volume increase (RVI) in shrunken cells involves the activation of a Na^+-H_+ exchange system [14,15]. On the other hand, in other cells alternative mechanisms have been invoked [1–5]. For the regulatory volume decrease these include KCl cotransport in sheep red cells [16] and in ascites cells ** [17], and a

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** An alternative mechanism for RVD in ascites cells involving conductive K^+ and Cl^- pathways has been recently described by Hoffmann et al. [23]. This mechanism is essentially identical to that of lymphocytes.

K^+-H^+ exchange in nucleated red cells of fish and amphibians [5]. For the regulatory volume increase a NaCl cotransport in ascites cells [18] and a Na^+-K^+-Cl^- cotransport in bird red blood cells [19,20] have been proposed. The number of cell types and species so far studied is quite limited and no clear pattern of volume-activated transport pathways has emerged.

In the case of mammalian cells osmotic volume regulation has been extensively studied only in lymphocytes [6–14] and Ehrlich ascites cells [17,18,21–23]. For the present study we selected the chinese hamster ovary (CHO) cell because it offered particular advantages: (1) It is an established line of transformed cells, many of its characteristics being well described; (2) it is easy to grow in quantity; (3) it is of fibroblast origin and is different in its state of differentiation to lymphoid or ascites cells; (4) it can grow in suspension so that volume changes can be accurately and easily measured by electronic sizing techniques (using the Coulter Counter), and the membrane potentials by fluorometric techniques; (5) it offers the potential for the study of transport mutants, because of relative ease of mutagenization and selection of mutants, and also because of the considerable body of existing genetic information about CHO cells (see Ref. 24).

In the present study CHO cells were subjected to hypo- and hyperosmotic media and their capacity to regulate their volumes was assessed. Using a number of techniques developed for lymphoid cells, the underlying mechanisms were characterized. The results allow comparisons of volume regulatory mechanisms of CHO, lymphoid, and other cell types.

Materials and Methods

Gramicidin, oligomycin C, quinine, ouabain and monensin were obtained from SIGMA Chemical Co. Amiloride was from Merck Sharp and Dohme, cetiedil was a gift of Dr. L. Berkowitz, University of North Carolina, Chapel Hill, bumetanide that of Lovens Kemiske Fabrik, Denmark, and diS-C_3-(5) of Dr. A. Waggoner, Amherst College, MA, U.S.A. The isotopes $^{86}Rb^+$ and $^{36}Cl^-$ were from Amersham.

CHO cells (wild type, Toronto strain), were obtained from Dr. L. Siminovitch, The Hospital for Sick Children. The cells were cultured in suspension and harvested by centrifugation [25]. Viability of the cells was assessed by Trypan blue dye exclusion and was invariably higher than 85%. The standard incubation solution contained 103 mM NaCl/5.4 mM KCl/23.8 mM $NaHCO_3$/5.6 mM Na_2HPO_4/0.4 mM $MgSO_4$/0.04 mM $CaCl_2$/10 mM glucose/10 mM Hepes (pH 7.1). Where indicated in the text Na^+ was replaced by the same concentrations of K^+ or $Tris^+$.

Cell volume changes were followed by electronic sizing using a Coulter Counter as described for lymphocyte studies [9,10]. All the experiments reported were repeated at least three times with different batches of cells. The temperature was 22°C, unless indicated otherwise.

The mechanisms underlying volume responses to anisotonic media were evaluated by procedures previously developed in similar studies with lymphocytes. In these cells, after osmotic swelling, substantial increases in conductive K^+ and Cl^- permeabilities allow the rapid gain or loss of KCl and osmotically obliged water depending on the direction of the K^+ plus Cl^- gradient [9–11]. The limiting factor appears to be the K^+ permeability so that the addition of the cation ionophore gramicidin, to increase K^+ permeability, results in faster volume changes [11,12]. Under these conditions the rate of volume change is no longer limited by the K^+ permeability but reflects the conductive anion permeability [11,12]. Replacement of Na^+ by $choline^+$ or $Tris^+$, whose permeation is not affected by gramicidin, results in an increased rate of shrinking; in Na^+ or K^+ media gramicidin produces marked swelling. Drugs that block the volume changes in the absence but not in the presence of gramicidin can be considered to inhibit the volume-activated K^+ permeability. Drugs that inhibit in the absence or presence of gramicidin can be considered either to specifically inhibit the volume-activated Cl^- permeability, or to inhibit the Cl^- and K^+ permeabilities to the same extent. These alternatives can be tested by independently assessing the effects of the inhibitors on K^+ and Cl^- fluxes following hypoosmotic stress, using isotope techniques ($^{86}Rb^+$ for K^+ and $^{36}Cl^-$ for Cl^-) as previously described [10–12].

Under certain conditions lymphocytes, after

osmotic shrinking, regain their normal volume by a mechanism involving activation of an electroneutral Na^+-H^+ exchange [14]. This mechanism can also be activated by acidification of the cytoplasm [15]. The presence of this exchange system can be simply demonstrated by measurement of volume changes following replacement of Cl^- in the normal NaCl medium by propionate (Grinstein et al. (1984) in the press). Undissociated propionic acid is highly lipid soluble and thus rapidly penetrates the cell membrane, while propionate anion has a comparatively low permeability [30–32]. The uptake of the free acid will produce an intracellular acidification, the magnitude of which depends on the propionic acid concentration and on the buffering capacity of the cell interior. If the acidification activates a Na^+-H^+ exchange, then the process will continue with a resultant net uptake of sodium propionate and a corresponding cell swelling that can be monitored by the Coulter Counter technique. The swelling is inhibited by amiloride, an inhibitor of the Na^+-H^+ exchange system.

Membrane potential in CHO cells was assessed by measuring fluorescence of the dye diS-C_3-(5), as described in Refs. 11, 13 and 26. Briefly, $(1–2) \cdot 10^6$ cells were incubated in a cuvette containing 1.0 ml incubation medium. The fluorescent dye was added in a final concentration of 0.5 μM and the fluorescence signals were recorded, with excitation and emission wavelengths of 620 and 670 nm, respectively, in a Perkin-Elmer Spectrofluorometer. This lipid-soluble dye reports the potential because its distribution between the cells and the medium is modulated by the membrane potential and because its fluorescence when inside the cells is largely quenched [26,28]. After dye equilibration an increase in fluorescence reports depolarization and a decrease indicates hyperpolarization. The membrane potential of CHO cells in isoosmotic media was estimated by the null-point method using valinomycin (a specific K^+ ionophore) at varying extracellular K^+ concentrations (see Refs. 11 and 26). Valinomycin at a concentration of 1 μM was found to produce a maximum response.

Cell Na^+ and K^+ contents were measured by flame-photometry [27], after washing CHO cells twice in large volumes of cold Na^+- and K^+-free media (Tris-HCl, pH 7.1), and resuspending and disrupting the cells by freezing and thawing in 15 mM LiCl.

For scanning electron microscopy the cells were fixed in the incubation media by the addition of glutaraldehyde (final concentration: 1%). After fixation for 2 h at room temperature the cells were washed three times with isoosmotic phosphate buffer and then prepared for microscopy.

Results

1. Regulatory volume decrease (RVD)

CHO cells suspended in 0.5 × or 0.3 × isoosmotic media (50% or 70% dilution with distilled water) first swell and then shrink towards their isoosmotic volumes (Fig. 1), thus showing a regulatory volume decrease. Oligomycin C (2 μg/ml) completely inhibits the regulatory volume decrease in CHO cells (Fig. 1). Similar results were obtained with quinine (50–100 μM), cetiedil (5–20 μM), dipyridamole (100 μM) and trifluoperazine (5–20 μM). On the other hand, ouabain (0.2 mM) or bumetanide (0.1 mM) have no effect on these volume changes.

The initial swelling reflects the uptake of water driven by the imposed osmotic gradient (Fig. 1). To assess the osmotic swelling, CHO cells were kept in media with various osmolarities for 20 min, in the absence or presence of oligomycin C, respectively. The resulting data are presented on a Van't Hoff plot (relative cell volume plotted against 1/osmolarity of the media, Fig. 2). In hyperosmotic media there was no difference between the volumes of the control and the drug-treated cells. In hypoosmotic media, however, large differences were evident. The control cells were found to be at about isoosmotic volumes regardless of the amount of dilution, due to the completion of the regulatory volume decrease response. In contrast, the oligomycin-treated cells, in which the regulatory volume decrease was inhibited, behaved essentially as osmometers, with the data points falling along a straight line. The intercept of this line with the ordinate indicates that about 20% of the original cell volume is osmotically non-responsive.

In order to follow the morphological changes of the CHO cell surface during volume regulation, cells were fixed with glutaraldehyde both before and 1 min or 20 min after hypoosmotic dilution.

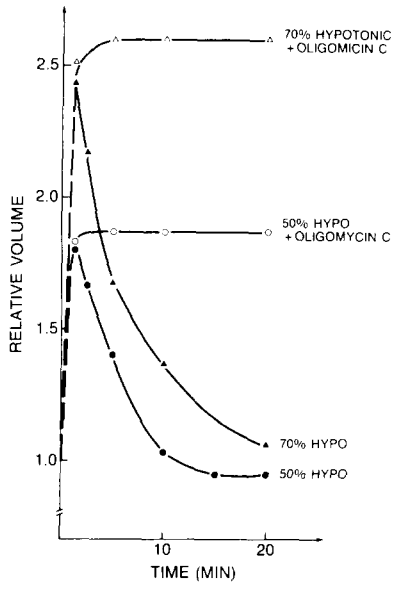

Fig. 1. Regulatory volume decrease of CHO cells in hypoosmotic media. CHO cells (10^5/ml) were placed in media diluted with 50% or 70% distilled water and volume changes were followed by a Coulter Counter as described in Methods. ●, 50% hypoosmotic media, ±0.2 mM ouabain; ○, 50% hypoosmotic medium + 2 μg/ml oligomycin C; ▲, 70% hypoosmotic media, ±0.2 mM ouabain; △, 70% hypoosmotic medium + 2 μg/ml oligomycin C.

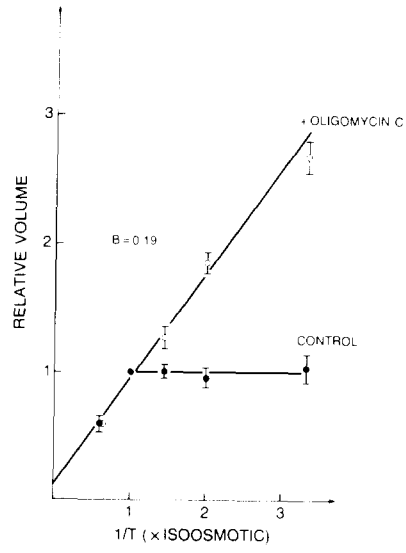

Fig. 2. Van't Hoff plot of the volumes of CHO cells recorded 20 min after suspension in media with various osmolarities. ●, control cells; ○, + 2 μg/ml oligomycin C. The slope of the line (0.786) and the B value (0.19) were calculated by linear regression using the means of 4–6 experiments for each data point. Points are mean ± S.D.

As shown in Fig. 3, cell swelling in hypoosmotic media produces the disappearance of the numerous membrane evaginations seen in the isoosmotic cells. The large amount of membrane folding apparent in the isoosmotic CHO cells is the probable explanation of the capacity of these cells to tolerate exposure to a dilution of as much as 70% (0.3 × isoosmotic) with no evidence of lysis and a full capacity to undergo regulatory volume decrease. As the cell swells, the membrane folds disappear. Lysis presumably occurs only if swelling is sufficient to fully unfold and stretch the membrane.

After volume restoration by regulatory volume

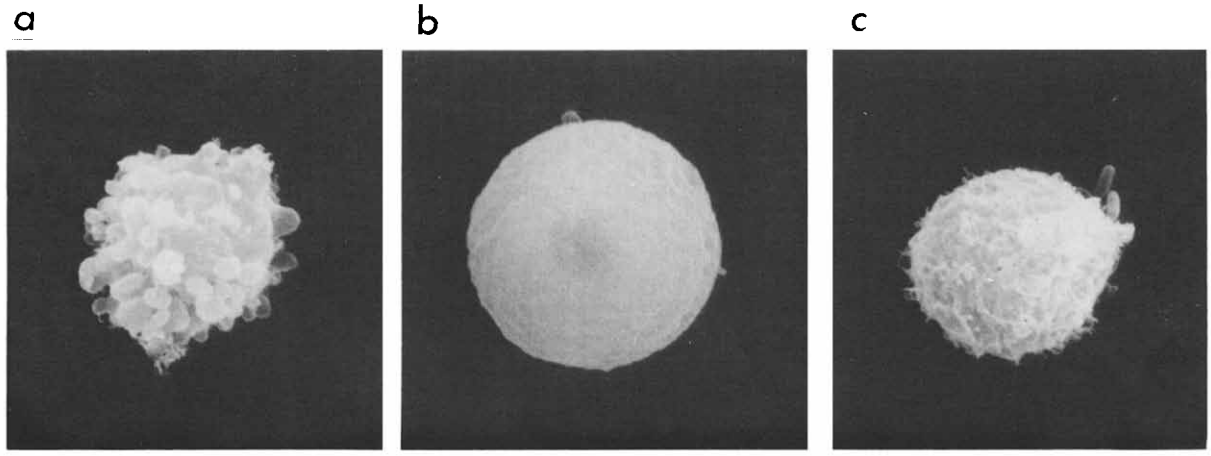

Fig. 3. Scanning electromicrographs of CHO cells fixed with glutaraldehyde (see Methods). (A) In isoosmotic media; (B) in 70% hypoosmotic media after 1 min incubation; (C) in 70% hypoosmotic media after 20 min incubation. Magnification = 5400×.

decrease the CHO cell surface is quite different from the original state: the protuberances are less numerous and their size much smaller (Fig. 3). This phenomenon is probably related to the appearance of a number of small vesicles (visible by electron microscopy) in the cell suspension after completion of the regulatory volume decrease. It appears as if some of the folded membrane parts had pinched off the cell surface during the regulatory volume decrease. Nevertheless, the cells are viable in terms of vital dye exclusion and capacity to grow.

The regulatory volume decrease response is associated primarily with a loss of cellular K^+ (Table I). K^+ and Na^+ ion contents in CHO cells were measured before and after volume regulation in 0.5 × isoosmotic media. As shown, control cells have a high K^+ and low Na^+ content (K^+/Na^+ is about 4.4 : 1). After volume regulation in hypoosmotic media, however, the total monovalent cation content was reduced by over 40%, with most of the loss (over 90%) accounted for by K^+. In the presence of ouabain, which inhibits the Na^+-K^+ pump but not the regulatory volume decrease, the pattern was similar, but K^+ loss was somewhat greater and Na^+ loss somewhat smaller. The K^+/Na^+ ratio in this case was reduced to 2.1 : 1. These results suggest that regulatory volume decrease involves primarily a loss of K^+ (and associated anion) by a mechanism independent of the Na^+-K^+ pump. The activity of the pump results, within the time of the experiment, in some readjustment of Na^+ and K^+ contents, so that a small amount of Na^+ appears to be lost during regulatory volume decrease. In the presence of ouabain this secondary loss of Na^+ is blocked.

TABLE I

CHANGES IN MONOVALENT CATION CONTENTS IN CHO CELLS DURING REGULATORY VOLUME DECREASE IN HYPOOSMOTIC MEDIA

Cation contents were measured by flame photometry as described in Methods. Figures are presented as mean ± S.D. values, $n = 5$. The median volume of CHO cells, as measured by the Coulter Counter, was $(1.25–1.30)\cdot 10^{-12}$ l.

	Cation contents (nmol/10^6 cells)		
	Isoosmotic control	15 min after hypoosmotic (0.5 × iso) shock	
		Control	+ 0.2 mM ouabain
K^+	200.7 ± 10.7	112.9 ± 8.4	98.2 ± 7.3
Na^+	46.1 ± 8.6	34.6 ± 3.3	41.8 ± 4.3

The addition of gramicidin significantly facilitates regulatory volume decrease (Fig. 4). As noted in the methods, the experiment is carried out in a Tris-HCl rather than a NaCl medium, a substitution which does not influence the regulatory volume decrease. It is important to note that gramicidin does not produce volume changes in CHO cells suspended in isotonic medium, indicating that under these conditions the anion conductance is relatively low. In the absence of gramicidin, quinine is an effective inhibitor of regulatory volume decrease, but in the presence of the ionophore inhibition does not occur. This finding indicates that quinine is a specific inhibitor of the

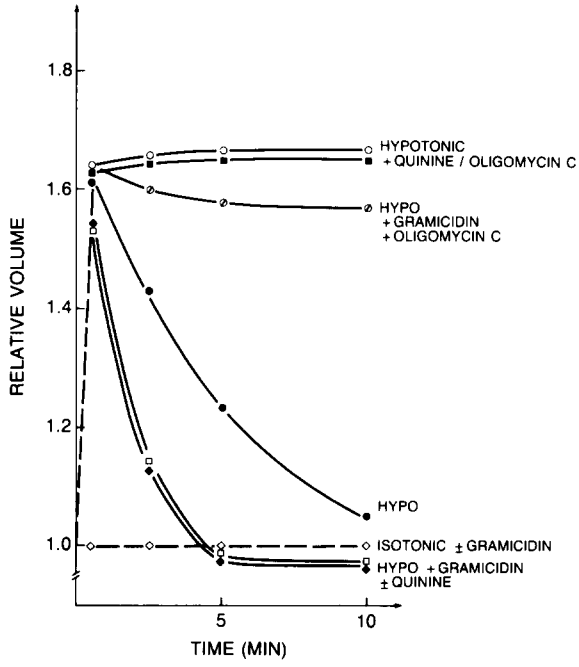

Fig. 4. Effects of drugs on regulatory volume decrease of CHO cells in hypoosmotic Tris-Hepes media. CHO cells were incubated in isoosmotic or 0.5 × isoosmotic media containing $Tris^+$ as the major cation. ◇, isoosmotic medium ± 1 μM gramicidin; ●, hypoosmotic medium, control; ◆, hypoosmotic medium + 1 μM gramicidin; ■, hypoosmotic medium + 0.1 mM quinine; □, hypoosmotic medium + 0.1 mM quinine + 1 μM gramicidin; ○, hypoosmotic medium + 2 μg/ml oligomycin C; ∅, hypoosmotic medium + 2 μg/ml oligomycin C + 1 μM gramicidin.

volume-activated K^+ efflux but if the quinine-blocked pathway is by-passed by gramicidin, the regulatory volume decrease can proceed [12]. On the other hand, oligomycin C inhibits regulatory volume decrease both in the presence and absence of gramicidin. Because cation permeation through the gramicidin channel is not affected by oligomycin (Ref. 12, and see also the discussion of Fig. 6 below), it appears that oligomycin C blocks the regulatory volume decrease by inhibiting the volume-activated anion permeability.

In hypoosmotic K^+-rich medium, the initial osmotic swelling is followed by a secondary swelling (rather than regulatory volume decrease) due to the net inward gradient for K^+ plus Cl^- (see Methods), as illustrated in Fig. 5. The secondary swelling also occurs with NO_3^- substitution (not shown) and is somewhat faster with SCN^-, but is minimal with gluconate$^-$, an impermeant anion. Addition of gramicidin further increased the rate and magnitude of the secondary swelling (Fig. 5) presumably by increasing the K^+ permeability of the cell membrane. The secondary hypoosmotic swelling was inhibited by oligomycin either in the presence or absence of gramicidin.

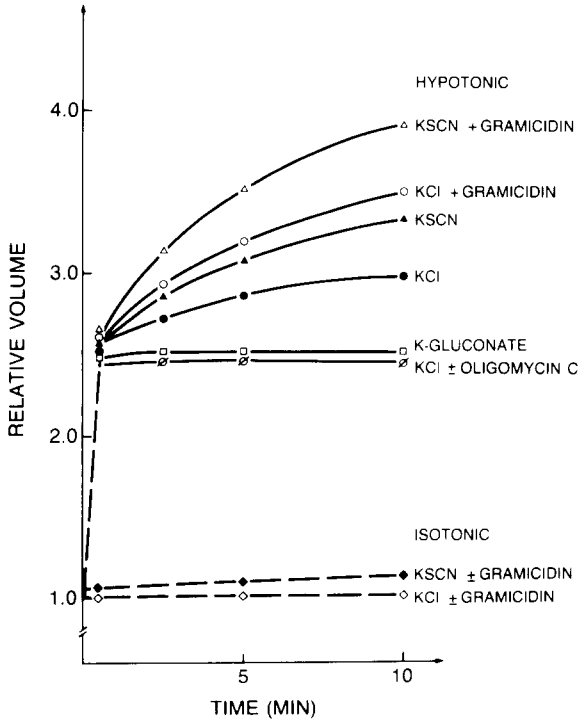

Fig. 5. Volume changes in CHO cells in isoosmotic or 0.3× isoosmotic media containing K^+ as the major cation. ◇, isoosmotic KCl medium, ±1 μM gramicidin; ◆, isoosmotic KSCN medium, ±1 μM gramicidin; ●, hypoosmotic KCl medium; ○, hypoosmotic KCl medium + 1 μM gramicidin; ▲, hypoosmotic KSCN medium; △, hypoosmotic KSCN medium + 1 μM gramicidin; □, hypoosmotic potassium gluconate medium, ±1 μM gramicidin; ∅, hypoosmotic KCl medium + 2 μg/ml oligomycin C.

The changes in the membrane potential of CHO cells during regulatory volume decrease were followed using the fluorescent dye diS-C_3-(5). The value of the membrane potential in resting CHO cells, determined by the valinomycin null-point procedure (see Methods), assuming an intracellular K^+ concentration of 160 mM (see Table I), was estimated to be −60 to −65 mV. As shown in Fig. 6 (part A), hypoosmotic dilution of CHO cells in NaCl media produces a rapid depolarization of the cell membrane, which is not observed during isoosmotic dilution. This depolarization presumably reflects the increased Cl^- conductance which, as the previous experiments indicated (Figs. 4 and 5), exceeds K^+ conductance in hypoosmotically shocked cells. Oligomycin C strongly inhibits the volume-induced depolarization of the CHO cell membrane, as expected from its blocking effect on the increased anion conductance during regulatory volume decrease. Addition of gramicidin in NaCl media produces a maximum depolarization of the cell membrane by a non-selective increase of both K^+ and Na^+ conductances. Oligomycin C does not affect the gramicidin-induced depolarization, excluding a direct interaction between these two agents.

In Tris-HCl media, hypoosmotic dilution results in a similar depolarization of the cell membrane to that produced in NaCl media (Fig. 6, part B). This absence of cation-dependence supports the conclusion that the observed depolarization results from a large increase in anion conductance during regulatory volume decrease. Addition of gramicidin to cells in hypoosmotic Tris-HCl medium reverses the volume-induced depolarization and causes a small hyperpolarization in isoosmotic cells. Such an effect is expected given that in the presence of the ionophore cation conductances exceed anion conductances, and that the gradient for permeating cations is almost entirely outward because gramicicin does not increase the permeability to $Tris^+$.

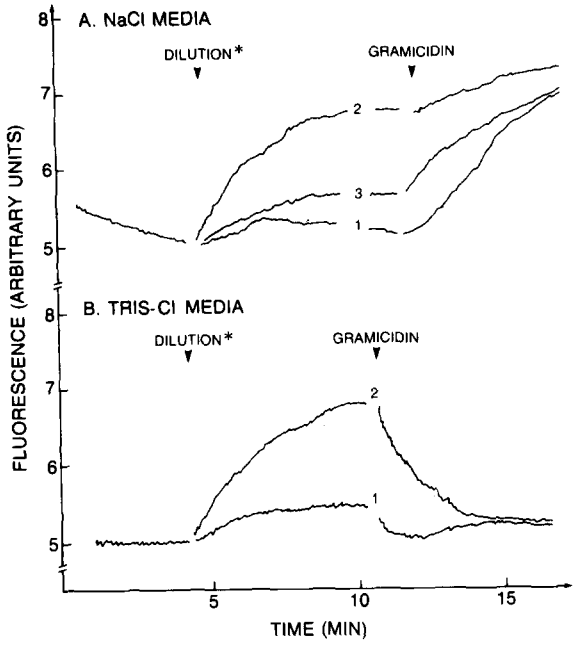

Fig. 6. Changes in membrane potential of CHO cells upon hypoosmotic dilution of the incubation media. Membrane potential was measured with the cationic carbocyanine dye, diS-C_3-(5), as described in Methods. (A) NaCl media; (B) Tris-HCl media. * Immediately after dilution of the cells with isoosmotic media or distilled water (resulting in a 50% hypoosmotic shock) the recorder scale was adjusted to the same relative fluorescence (i.e. 5 units). (1) isoosmotic dilution; (2) 50% hypoosmotic dilution; (3) 50% hypoosmotic dilution + oligomycin C (2 μg/ml). Where indicated, gramicidin (1 μM) was added.

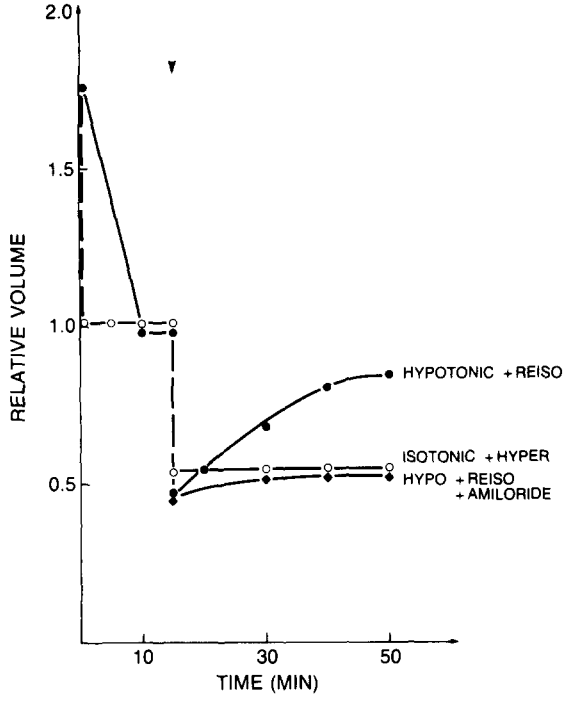

Fig. 7. Regulatory volume decrease and regulatory volume increase in CHO cells. Temperature 37°C. ○, isoosmotic medium, adjusted to 2.0×isoosmotic with concentrated NaCl at the time indicated by the arrow; ●, 50% hypoosmotic medium, readjusted to isoosmotic with concentrated NaCl at the time indicated by the arrow; ◆, as the latter, +100 μM amiloride.

2. Regulatory volume increase (RVI)

CHO cells placed directly into hyperosmotic media behave as osmometers, without evidence of volume-regulatory activity (Figs. 2 and 7). On the other hand, CHO cells first exposed to a hypoosmotic shock and transferred to an isoosmotic NaCl medium after regulatory volume decrease had been completed, shrink below the isoosmotic volume (due to the previous loss of salts during regulatory volume decrease) and then show a regulatory volume increase (RVI). This regulatory volume increase was abolished by 100 *M* amiloride (Fig. 7), a known inhibitor of Na^+ transport pathways (see Ref. 29). If hypoosmotic shock and the restoration of isoosmotic conditions are carried out in Tris-HCl media, regulatory volume decrease is normal but no regulatory volume increase is observed. The same pattern is found with bicarbonate-free NaCl media (data not shown). From these experiments it is apparent that CHO cells have a Na^+-dependent amiloride-sensitive transport system, which in the presence of bicarbonate is capable of producing a regulatory volume increase.

In lymphocytes a similar phenomenon was documented to involve the parallel functioning of a Na^+-H^+ and a Cl^--HCO_3^- exchange system [14]. Furthermore, in those cells the Na^+-H^+ exchange could also be activated by intracellular acidification [15]. In order to determine whether a Na^+-H^+ exchange system is present in CHO cells, incubations were carried out in isoosmotic media containing Na^+ or K^+ plus a weak electrolyte, propionate, as the major anion, a procedure outlined in the Methods section. In Na^+ propionate medium, a rapid swelling was observed that was

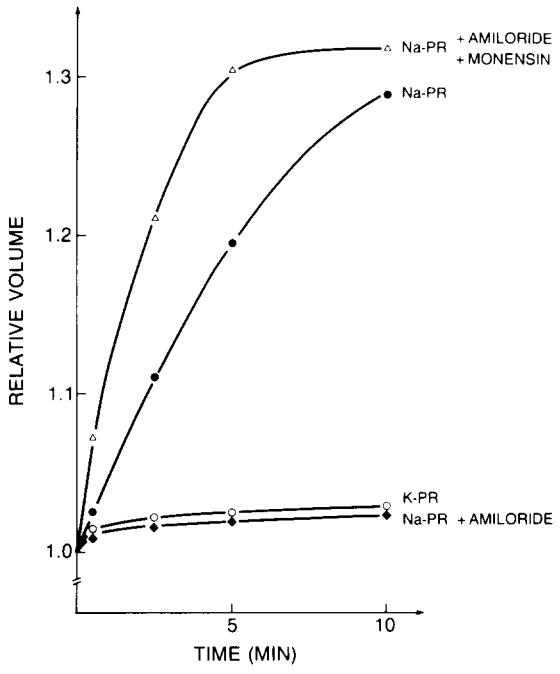

Fig. 8. Volume changes in CHO cells incubated in media containing propionate as predominant anion. The media contained 140 mM sodium or potassium propionate, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM glucose and 20 mM Tris-HCl, pH 6.8. Temperature 37°C. ●, Sodium propionate medium; ○, potassium propionate medium; ◆, Sodium propionate + 100 μM amiloride; ◇, potassium propionate + 100 μM amiloride + 2 μM monensin.

blocked by 100 μM amiloride or by substitution of K^+ for Na^+ (Fig. 8). The system was tested by addition of the ionophores monensin (which mediates Na^+-H^+ exchange) and nigericin (which mediates K^+-H^+ exchange). With monensin little volume change occurred in NaCl medium (data not shown), but in sodium propionate medium a substantial amiloride-insensitive swelling was observed. With nigericin, a rapid swelling was observed in potassium propionate but not KCl medium (not shown). These experiments strongly suggest that intracellular acidification activates a specific amiloride-sensitive Na^+-H^+ exchange pathway in CHO cells. In the presence of a permeant organic acid, activation of the exchange pathway results in rapid volume changes.

Attempts to directly measure the intracellular pH with carboxyfluorescein (see Ref. 34) in the above experiments were unsuccessful because the dye leaked rapidly out from CHO cells.

Discussion

The behaviour of CHO cells toward anisoosmotic media is similar in almost all respects to that of human T lymphocytes. In hypoosmotic media an initial rapid swelling is followed by a slower return toward normal, isoosmotic size (regulatory volume decrease). The swelling phase appears to be an equilibration of water driven by the imposed osmotic gradient. As in lymphocytes [6,9] the degree of swelling in CHO cells (when regulatory volume decrease is blocked) follows the Van't Hoff relationship (Fig. 2) indicating simple osmometric behaviour. The non-solvent volume (the intercept on the *Y* axis) is, however, less in CHO cells than in lymphocytes (20% compared to 32%). The rate of regulatory volume decrease is about the same in the two types of cells (complete within 10 min at room temperature, with 30% hypoosmotic shock). Furthermore, the same underlying mechanisms appear to be operative, namely volume-activated, electrogenic K^+ and Cl^- permeation pathways. The data on ion and inhibitor specificities suggest that the pathways are identical. The only significant difference seems to be in the magnitude of hypoosmotic dilution to which lymphocytes or CHO cells can respond with regulatory volume decrease. CHO cells, probably because of the large amount of folded membrane on the cell surface (see Fig. 3), behave as osmometers when regulatory volume decrease is blocked and respond with a fully active regulatory volume decrease in solutions as dilute as 0.3 × isoosmotic, while lymphocytes are damaged and lose their response at such dilutions [9].

The responses to hyperosmotic media are also similar in lymphocytes [14,15] and CHO cells. Direct exposure to hyperosmotic solutions results in osmotic shrinking in both cell types with no regulatory volume increase. On the other hand, after a cycle of regulatory volume decrease and reexposure to isoosmotic media, the shrinking phase is followed by reswelling (see Ref. 14 and Fig. 7). The mechanism involves volume-activated, amiloride-sensitive Na^+-H^+ exchange presumably coupled with a Cl^--HCO_3^- exchange. In both types of cells Na^+-H^+ exchange can also be activated by acidification of the cytoplasm (Ref. 15 and Fig. 8). The exact role of a previous

regulatory volume decrease cycle in obtaining an regulatory volume increase is still not entirely known, but the most plausible explanation is the requirement for Cl^- depletion.

It is rather striking that two cells of such a different lineage and differentiation should respond to anisoosmotic media in such a similar fashion, by the activation of three transport pathways (conductive K^+ and Cl^- pathways and a Na^+-H^+ exchanger). This pattern does not, however, apply to all mammalian cells as, for example, in B lymphocytes regulatory volume decrease is minimal, due to the failure of activation of the volume-induced K^+ transport. The Cl^- pathway, however, is fully activated in these cells by hypoosmotic shock [35,36]. Mammalian erythrocytes have little or no capacity to regulate their volumes, although in sheep red cells some regulatory volume decrease capacity has been noted, probably involving KCl cotransport [16]. In Ehrlich ascites cells regulatory volume decrease has been reported to involve similar mechanisms to those discussed above for lymphocytes and CHO cells [23]. On the other hand, a non-conductive KCl cotransport system has also been proposed [17]. Regulatory volume increase in ascites cells seems to be different from that in lymphocytes or CHO cells: a bumetanide-sensitive NaCl cotransport is postulated to be the underlying mechanism [18].

In non-mammalian cells, as well, no general pattern emerges. In red cells of amphibia and certain fish, regulatory volume decrease is reported to involve a K^+-H^+ exchanger, and regulatory volume increase a Na^+-H^+ exchanger, both in parallel to a Cl^--HCO_3^- exchanger system [5]. The latter mechanism also underlies regulatory volume increase in gall bladder epithelial cells of an amphibian [4]. Thus regulatory volume decrease seems to be different, while regulatory volume increase is similar to that in lymphocytes or CHO cells. In bird red cells the regulatory volume increase appears to involve a Na^+-K^+-Cl^- cotransport [19,20], while the mechanism of regulatory volume decrease has not yet been clearly established [2]. Clearly more types of cells need to be investigated in a definitive manner in order to establish the generalities of the pathways of osmotic volume regulation.

The three transport systems that can be activated by volume changes in lymphoid and CHO cells (K^+, Cl^-, and Na^+-H^+ exchange pathways) appear to be quiescent in normal, isoosmotic cells. Nevertheless, similar transport systems have been noted in other cells in connection with different functional activities. For example, the Na^+-H^+ exchange system seems to be involved in intracellular pH regulation and in the hormonal control or mitogenic stimulation of various cells [37–39]. The Ca^{2+}-activated K^+ pathways have been shown to produce functionally important hyperpolarizatoins in excitable as well as in non-excitable cells (see Refs. 40–42). It will require further efforts to determine the general prevalence of particular volume-controlling mechanisms in various mammalian cell types and to understand the relationship between volume-induced transport pathways and similar or identical systems involved in other kinds of membrane functions.

An objective of the present study was to establish the mechanism of volume regulation in a cell type that offers the potential of selecting and studying membrane transport mutants. For this purpose the CHO cell has many advantages. It can be used as a suspended cell, allowing the use of technologies, such as cell sizing with the Coulter Counter or potential measurements by the fluorescent-dye technique. On the other hand it will grow in the attached state, an advantage for the selection and cloning of mutants. Furthermore, many mutations have been already mapped, and the relevant techniques elaborated (see Ref. 24). Specific inhibitors of each of the volume-activated transport pathways have been identified, allowing the search for inhibitor-resistant mutants as well. The enormous increases in cell volume such as produced by hypoosmotic K^+-rich media (Fig. 5) may allow establishment of 'suicide' techniques to eliminate volume-responsive cells.

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References

1 MacKnight, A.D.C. and Leaf, A. (1977) Physiol. Rev. 57, 510–573
2 Kregenow, F.M. (1981) Annu. Rev. Physiol. 43, 493–505
3 Hoffmann, E.K. (1982) Phil. Trans. R. Soc. London B. 299, 519–535
4 Ericson, A. and Spring, K.R. (1982) Am. J. Physiol. 243, C146–C150
5 Cala, P.M. (1983) Mol. Physiol. 4, 33–52
6 Grinstein, S., Rothstein, A., Sarkadi, B. and Gelfand, E.W. (1984) Am. J. Physiol. 246, C204–C215
7 Roti-Roti, L.W. and Rothstein, A. (1973) Exptl. Cell. Res. 79, 295–310
8 Bui, A.H. and Wiley, J.S. (1981) J. Cell. Physiol. 108, 47–54
9 Cheung, R.K., Grinstein, S., Dosch, H.M. and Gelfand, E.W. (1982) J. Cell Physiol. 12, 189–196
10 Grinstein, S., DuPré, A. and Rothstein, A. (1982) J. Gen. Physiol. 79, 849–868
11 Grinstein, S., Clarke, C.A., DuPré, A. and Rothstein, A. (1982) J. Gen. Physiol. 80, 801–823
12 Sarkadi, B., Mack, E. and Rothstein, A. (1983) J. Gen. Physiol. 83, 497–512
13 Sarkadi, B., Mack, E. and Rothstein, A. (1983) J. Gen. Physiol. 83, 513–527
14 Grinstein, S., Clarke, C.A. and Rothstein, A. (1983) J. Gen. Physiol. 82, 619–638
15 Grinstein, S., Cohen, S. and Rothstein, A. (1983) J. Gen. Physiol. 83, 341–369
16 Ellory, J.C., Dunham, P.B., Logue, P.J. and Stewart, G.W. (1982) Phil. Trans. Roy. Soc. London B. 299, 483–495
17 Thornhill, W.B., Paxman, D., Zarka, M., Henius, G.V. and Laris, P.C. (1982) Fed. Proc. 41, 6434
18 Hoffmann, E.K., Sjoholm, C. and Simonsen, L.O. (1983) J. Membrane Biol. 76, 269–280
19 Schmidt, W.F. and McManus, T.J. (1977) J. Gen. Physiol. 70, 59–70
20 Haas, M., Schmidt, W.F. and McManus, T.J. (1982) J. Gen. Physiol. 80, 125–147
21 Hoffmann, E.K. (1978) in Alfred Benson Symp. XI. (Jørgensen, C.B. and Skadhauge, E., eds.), pp. 397–417, Munksgaard, Copenhagen
22 Hoffmann, E.K. (1982) Phil. Trans. R. Soc. London B. 299, 519–535
23 Hoffmann, E.K., Simonsen, L.O. and Lambert, I.H. (1983) J. Membrane Biol. 78, 211–223
24 Ray, P.N. and Siminovitch, L. 91982) in Somatic Cell Genetics (Casley, T. and Robbins, P., eds.), pp. 127–167, Plenum Publishing Co., New York
25 Thomson, L.H. and Baker, R.M. (1973) Methods Cell Biol. 6, 209–281
26 Waggoner, A.S. (1979) Methods Enzymol. 55, 689–695
27 Graves, J.S. and Wheeler, D.D. (1982) Am. J. Physiol. 243, C124–C132
28 Hladky, S.B. and Rink, T.J. (1976) J. Physiol. 263, 287–319
29 Benos, D.J. (1982) Am. J. Physiol. 242, C131–C145
30 DeHemptinne, A., Marrannes, R. and Vanhel, B. (1983) Am. J. Physiol. 245, C178–C183
31 Keifer, D.W. (1981) Am. J. Physiol. 241, C193–C199
32 Rindler, M.J. and Saier, M.H. (1981) J. Biol. Chem. 256, 10820–10824
33 Pressman, B.C. (1976) Annu. Rev. Biochem. 45, 501–530
34 Boron, W.F. (1983) J. Membrane Biol. 72, 1–16
35 Cheung, R.K., Grinstein, S. and Gelfand, E.W. (1982) J. Clin. Invest. 70, 632–638
36 Grinstein, S., Clarke, C.A., Rothstein, A. and Gelfand, E.W. (1983) Am. J. Physiol. 245, C160–C163
37 Rink, T.J., Tsien, R.Y. and Pozzan, T. (1982) J. Cell. Biol. 95, 189–196
38 Vigne, P., Frelin, C. and Lazdunski, M. (1982) J. Biol. Chem. 257, 9394–9400
39 Weinman, S.A. and Reuss, L. (1982) J. Gen. Physiol. 80, 299–321
40 Ferreira, H.G. and Lew, V.L. (1977) in Membrane Transport in Red Cells (Ellory, J.C. and Lew, V.L., eds.), pp. 53–92, Academic Press, New York
41 Schwarz, W. and Passow, H. (1983) Annu. Rev. Physiol. 45, 359–374
42 Sarkadi, B. and Gardos, G. (1984) in The Enzymes of Biological Membranes, 2nd Edn. (Martonosi, A., ed.), Plenum Publishing Co., New York, in the press