Biochimica et Biophysica Acta, 946 (1988) 397–404
Elsevier

BBA 74280

Characterization of $Na^+/K^+/Cl^-$ cotransport in cultured HT29 human colonic adenocarcinoma cells

Hyun Dju Kim, Yu-Shan Tsai, Christopher C. Franklin and John T. Turner

Department of Pharmacology, School of Medicine, University of Missouri-Columbia, Columbia, MO (U.S.A.)

(Received 4 May 1988)

Key words: cyclic AMP; Ion transport; ATP depletion; Sodium/potassium/chloride cotransport; (Stoichiometry)

A $Na^+/K^+/Cl^-$ cotransport pathway has been examined in the HT29 human colonic adenocarcinoma cell line using ^{86}Rb as the K congener. Ouabain-resistant bumetanide-sensitive (OR-BS) K^+ influx in attached HT29 cells was 17.9 ± 0.9 nmol/min per mg protein at 25°C. The identity of this pathway as a $Na^+/K^+/Cl^-$ cotransporter has been deduced from the following findings: (a) OR-BS K^+ influx ceased if the external Cl^- (Cl_o^-) was replaced by NO_3^- or the external Na^+ (Na_o^+) by choline; (b) neither OR-BS $^{24}Na^+$ nor $^{36}Cl^-$ influx was detectable in the absence of external K^+ (K_o^+); and (c) concomitant measurements of $^{86}Rb^+$, $^{22}Na^+$, and $^{36}Cl^-$ influx indicated that the stoichiometry of the cotransport system approached a ratio of $1N^+ : 1K^+ : 2Cl^-$. In addition, OR-BS K^+ influx was exquisitely sensitive to cellular ATP levels. Depletion of the normal ATP content of 35–40 nmol/mg protein to 10–15 nmol/mg protein, a concentration at which the ouabain-sensitive K^+ influx was unaffected, completely abolished K^+ cotransport. OR-BS K^+ influx was slightly reduced by the divalent cations Ca^{2+}, Ba^{2+}, Mg^{2+} and Mn^{2+}. Although changes in cell volume, whether shrinking or swelling, did not influence OR-BS K^+ influx, ouabain-sensitive K^+ influx was activated by cell swelling. As in T84 cells, we found that the OR-BS K^+ influx in HT29 cells was stimulated by exogenous cyclic AMP analogues and by augmented cyclic AMP content in response to vasoactive intestinal peptide, forskolin, norepinephrine and forskolin or prostaglandin E_1.

Introduction

A quaternary cotransport system for Na^+, K^+ and Cl^- has now been extensively investigated in both epithelial and non-epithelial cells [10,24]. Recent work on the cotransport pathway reveals common salient features which include a stringent interdependency of the transported species and a narrow substrate specificity [3,20]. Thus, K^+ influx by the cotransport pathway ceases in the absence of either Na^+ or Cl^-. Lithium has been found to be a poor substitute for sodium [11,32] and the anions which can participate in cotransport are limited to chloride or bromide [12,29]. Cotransport is inhibited by several loop diuretics including bumetanide and the less potent furosemide.

Recently, Haas et al. [12] have shown that ion flux by the cotransport pathway is not affected over a wide membrane potential range in duck red

Abbreviations: DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; FSK, forskolin; PGE_1, prostaglandin E_1; NE, norepinephrine; FSK, forskolin; FIP, vasoactive intestinal peptide; BSS, balanced salt solution, DMEM, Dulbecco's modified Eagle's medium; HPLC, high-performance liquid chromatography; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; O, ouabain; B, bumetanide; R, -resistant; S, -sensitive.

Correspondence: H.D. Kim, Department of Pharmacology, School of Medicine, University of Missouri-Columbia, Columbia, MO 65212, U.S.A.

cells treated with DIDS and valinomycin. Thus, in most, but not all cases [13], the cotransport pathway mediates the electrically neutral movement of ions with a stoichiometry of 1:1:2 for Na^+, K^+ and Cl^-, respectively [10]. The activity of the cotransport system is dependent not only on the net driving force established by the sum of chemical potential gradients of all participating ion species across the cell membrane [14], but also on effects elicited by the generation of intracellular second messengers. An intriguing finding is that increased levels of cyclic AMP cause an inhibition of $Na^+/K^+/Cl^-$ cotransport in some cells and a stimulation in others [9,26,29,30]. This diametric regulation has also been observed with cyclic GMP which is produced in response to atrial natriuretic factor [22,23,25]. In addition, phorbol esters, most likely acting through stimulation of protein kinase C, elicit either inhibition [21] or stimulation [27,31] of cotransport, depending on the cell type.

In intestinal epithelia, the $Na^+/K^+/Cl^-$ cotransport system has been implicated in transcellular salt transport. Recently, two cell lines derived from human colonic cancer cells, T84 and HT29, have been used as models for studying epithelial cells. While T84 cells have been extensively characterized with respect to membrane transport processes, including $Na^+/K^+/Cl^-$ cotransport [4], cyclic AMP-activated chloride transport [18] and cyclic AMP- and Ca^{2+}-activated K^+ transport [19], relatively little is known about the ion transport properties of HT29 cells. On the other hand, HT29 cells have been widely used for the identification and characterization of plasma membrane receptors for a variety of hormones and neurotransmitters, including insulin [7,8], vasoactive intestinal peptide (VIP) [1,33] and α_2-adrenergic agonists [2,34].

In this paper, we present evidence for the presence of a $Na^+/K^+/Cl^-$ cotransport pathway in HT29 cells. In addition, the possible regulation of the cotransporter by cyclic AMP was investigated by taking advantage of the wide range of cyclic AMP levels that can be produced in HT29 cells [15,16,34]. Both the elevation of intracellular cyclic AMP by VIP, forskolin (FSK), prostaglandin E_1 (PGE_1), or by sequential treatment with norepinephrine (NE) and forskolin and the addition of exogeneous cyclic AMP analogues resulted in a stimulation of cotransport in HT29 cells.

Materials and Methods

Cell culture

HT29 cells from a human colonic adenocarcinoma [6] were grown as previously described [8,34] in DMEM supplemented with 5% fetal bovine serum and 5% newborn bovine serum in 75-cm^2 tissue culture flasks in a humidified atmosphere of 5% CO_2/95% air. Confluent cultures were subcultured with 0.05% trypsin/0.01% EDTA into either 35-mm diameter tissue culture dishes or 24-well multiwell plates at a density of $5 \cdot 10^4$ cells per cm^2 and grown to confluence with the medium being replaced every other day. Confluent cultures were used on days 6 to 9 of growth with the final medium change being no longer than 24 h before use.

K^+-influx determinations

K^+ influx was measured using ^{86}Rb as a K congener in HT29 cells that had received fresh medium the previous day. Cells were washed twice with DMEM containing 20 mM Hepes buffer (pH 7.4) (DMEM-Hepes) and were maintained at room temperature in the same medium for 90 min. Cells were then washed with 0.15 M NaCl and either 0.9 ml (35-mm dishes) or 0.27 ml (24-well plates) of a balanced salt solution (BSS) composed of (mM): 5 KCl/136 NaCl/5 glucose/1 sodium phosphate/10 Tris-Hepes buffer (pH 7.4) which was added to each dish. Ouabain was added at a final concentration of 0.2 mM to one set of dishes and 0.2 mM ouabain plus 0.1 mM bumetanide was added to a second set, unless otherwise noted in the figure legends. The third set received carrier only (dimethyl sulfoxide, 0.2% v/v final concentration). All dishes were then preincubated for 10 min at room temperature. To initiate the flux measurements, either 0.1 ml (35-mm dishes) or 0.03 ml (24-well plates) of BSS containing ^{86}Rb was introduced to give a final radioactivity of 1–2 μCi per dish. At frequent intervals, the medium was removed by aspiration and the cultures were rapidly washed two or three times with ice-cold 0.15 M NaCl. As ^{86}Rb influx in the presence or absence of uptake inhibitors was found to be

linear up to 10 min (Fig. 1), subsequent measurements were made using a 5-min influx period. The cells were then lysed with 1 ml of 0.2 M NaOH, which was transferred to a scintillation vial along with a subsequent 1 ml distilled water wash. After neutralization with 0.2 ml of 1 M HCl and addition of 18 ml of Budget-Solve, the radioactivity was measured by liquid-scintillation spectroscopy. To determine the level of retained extracellular radioactivity, cells were washed and lysed immediately after ^{86}Rb exposure. These values, amounting to 0.2% of total radioactivity per dish, were routinely determined in each experiment and used for the correction of radioactivity associated with the cell pellet. All determinations were made in duplicate. Flux values are expressed per mg cellular protein as determined by the procedure of Lowry [17].

In experiments where ion composition was varied, the figure legends indicate specific changes. The stoichiometry of Na^+, K^+ and Cl^- cotransport was determined by measuring $^{24}Na^+$ (or $^{22}Na^+$), $^{86}Rb^+$ and $^{36}Cl^-$ influx, respectively. These measurements were carried out concurrently by the same procedure used for ^{86}Rb uptake except that a 1-min influx time period was used and the final radioactivity of the appropriate isotope was 2–3 μCi per dish.

Cell ATP determination

The cellular ATP content was determined according to the procedure described elsewhere [8]. Briefly, the cells were extracted with 0.3 ml of 2.5 M perchloric acid and the extract was neutralized to pH 6.9–7.3 with 6.5 M K_2CO_3. The ATP content of the perchloric acid extract was determined by HPLC.

Sources of materials

$^{86}Rb^+$, $^{22}Na^+$ and $^{36}Cl^-$ were purchased from New England Nuclear Corp. (Boston, MA). $^{24}Na^+$ was obtained from the University of Missouri Research Reactor facility. Furosemide was purchased from Aldrich Chemical Co. (Milwaukee, WI). Bumetanide was a generous gift from Dr. Peter Sorter of Hoffmann LaRoche Co. (Nutley, NJ). VIP was obtained from Peninsula laboratories (Bermont, CA). PGE_1, 2-deoxyglucose, 8-bromo-cyclic AMP and NE were purchased from Sigma Chemical Co. (St. Louis, MO) and FSK was purchased from Calbiochem-Behring (La Jolla, CA). Sera, trypsin and 24-well tissue culture plates were obtained from Gibco Laboratories (Grand Island, NY), DMEM was purchased from KC Biologicals Inc. (Lenexa, KS) and Falcon 35-mm tissue culture dishes were from Fisher Scientific Co. (St. Louis, MO). Budget-Solve was purchased from Research Products International Corp. (Mt. Prospect, IL).

Results

K^+ influx

The time-course of K^+ influx in the presence or absence of ouabain or ouabain and bumetanide in HT29 cells is shown in Fig. 1. Total K^+ influx was found to be linear up to 10 min. In preliminary experiments, it was determined that preincubation with ouabain for at least 10 min was necessary in

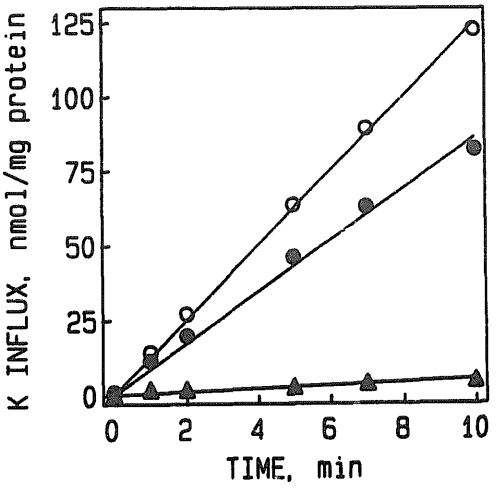

Fig. 1. Time-course of K^+ influx in the presence or absence of bumetanide and/or ouabain in HT29 cells. After removing the growth medium, HT29 cells were allowed to equilibrate in DMEM-Hepes buffer (pH 7.4) for 90 min at room temperature. Cells were then preincubated for 10 min without drug, with 0.2 mM ouabain or with 0.2 mM ouabain plus 0.1 mM bumetanide in a balanced salt solution (BSS) composed of (mM): 5 KCl/136 NaCl/5 glucose/1 sodium phosphate/10 Tris-Hepes buffer (pH 7.4). BSS containing $^{86}Rb^+$ was then added to give a final radioactivity of 1–2 μCi/dish. Uptake was stopped at the indicated times and the cell-associated radioactivity was determined. No inhibitors (open circles); ouabain (closed circles); ouabain plus bumetanide (triangles). Data shown are from a representative experiment performed in duplicate and repeated four times.

TABLE I

K^+ INFLUX IN HT29 CELLS

K^+ influx was measured in control cells as stated in the Materials and Methods. The values presented are the mean ± S.E. of the number of experiments shown in parentheses.

Influx pathway	nmol/min per mg protein
Ouabain-sensitive K^+ influx *	4.7 ± 0.6 ($n = 56$)
Ouabain-resistant bumetanide-sensitive K^+ influx	17.9 ± 0.9 ($n = 69$)

* As stated in the text, from 69 determinations 13 measurements, which exhibited no measurable OS K^+ influx, are excluded.

order to fully inhibit ouabain-sensitive (OS) K^+ influx. Since 0.2 mM ouabain was maximally effective in inhibiting OS K^+ influx (data not shown), we routinely used a 10-min preincubation with 0.2 mM ouabain in measuring $Na^+/K^+/Cl^-$ cotransport activity. For reasons which are not entirely clear, the magnitude of the OS K^+ influx was highly variable. Some HT29 cell passages had little or no measurable active K^+ influx. From 69 determinations, 13 measurements showed no detectable active K^+ influx (Table I). However, regardless of the magnitude of active K^+ influx, the addition of either bumetanide or furosemide to ouabain-pretreated cells resulted in a virtual abolition of K^+ influx in every case (Fig. 1), implying that K^+ entry is mediated to a large extent by the ouabain-resistant (OR) diuretic-sensitive pathway. The OR diuretic-insensitive component, which is presumed to reflect a leak pathway, represents a minute fraction of the total K^+ influx.

Diuretic dose–response relationship

As is the case for most cell types, bumetanide was more potent than furosemide in inhibiting OR diuretic-sensitive K^+ influx in HT29 cells. The half-maximal inhibitory concentrations of bumetanide and furosemide were approx. 0.5 μM and 7 μM, respectively, as shown in Fig. 2. In all subsequent experiments, 0.1 mM bumetanide was used to define OR diuretic-sensitive K^+ influx.

Effects of external, K^+, Na^+ and Cl^- on OR-BS K^+ influx

Fig. 3A shows OR-BS K^+ influx as a function of external K^+ (K_o^+). External Na^+ and Cl^- were held constant at 105 and 136 mM, respectively. External K^+ was varied by replacement with tetramethylammonium chloride. The V_{max} was 18.4 nmol/min per mg protein with a K_m for K^+ of 2.5 mM, similar to previously reported K_m values in other systems of 1.5–10 mM [3,13,20,26,28,35].

Figs. 3B and C show the absolute dependency of OR-BS K^+ influx on external Na^+ (Na_o^+) and Cl^- (Cl_o^-), respectively. When external Na^+ concentrations were varied, the isotonicity of BSS was maintained with tetramethylammonium chloride. With the media K^+ and Cl^- being held constant at 5 and 141 mM, respectively, raising the $[Na^+]_o$ resulted in a progressive increase in K^+ influx with saturation taking place near 25 mM (Fig. 3B). V_{max} was 10.7 nmol/min per mg protein with a K_m for Na^+ of 3.9 mM.

External chloride was varied with isotonicity maintained by replacement with NO_3^-. The media Na^+ and K^+ were held constant at 131 and 5 mM, respectively. As $[Cl^-]_o$ was raised, OR-BS K^+ influx increased linearly, with no apparent saturation up to 116 mM $[Cl^-]_o$ (Fig. 3C). A compara-

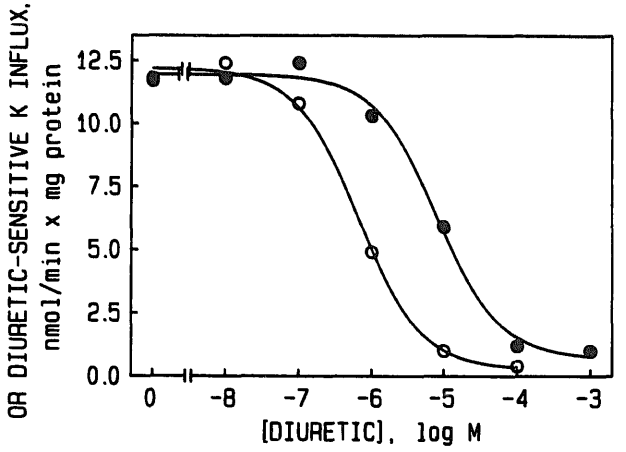

Fig. 2. Concentration-dependent inhibition of ouabain-resistant K^+ influx by loop diuretics in HT29 cells. The experimental conditions were similar to those described in Fig. 1, except that cells were preincubated for 10 min in BSS in the presence of 0.2 mM ouabain and the indicated concentrations of bumetanide (open circles) or furosemide (closed circles). K^+ uptake was determined after a 5-min influx period. The data shown are from a representative experiment performed in duplicate and repeated two times.

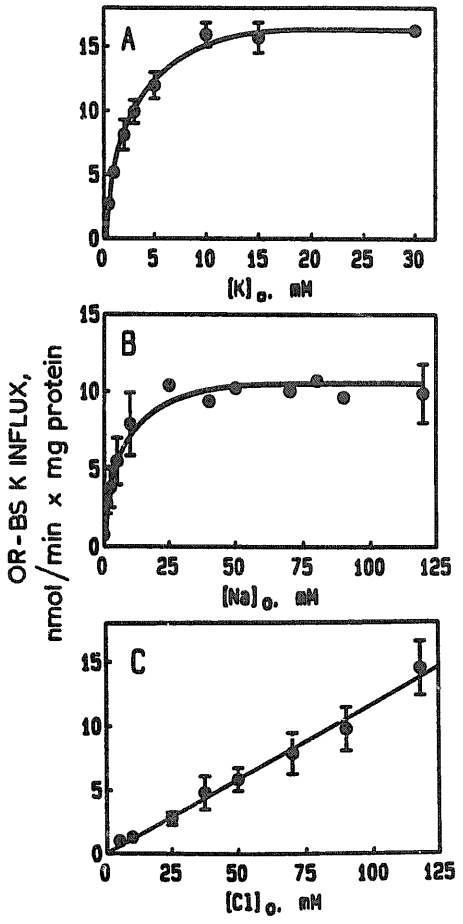

Fig. 3. Dependence of OR-BS K^+ influx on $[K^+]_o$, $[Na^+]_o$ and $[Cl^-]_o$. (A) Effect of external K^+ on OR-BS K^+ influx. The Na^+ and Cl^- content in BSS were reduced to 105 mM and 136 mM, respectively. The K^+ concentration was varied with the isotonicity and Cl^- content being maintained with tetramethylammonium chloride. Values shown are the mean ± S.E. of two or four experiments, each performed in duplicate. (B) Effect of external Na^+ on OR-BS K^+ influx. The medium composition was the same as in Fig. 1, except that the isotonicity and Cl^- content at the various Na^+ concentrations were maintained with tetramethylammonium chloride. Values shown are the mean ± S.E. of three or four experiments, each performed in duplicate. (C) Effect of external Cl^- on OR-BS K^+ influx. The Cl^- concentration in BSS was varied by replacement with NO_3^-, while the Na^+ and K^+ content were held constant at 131 mM and 5 mM, respectively. Values shown are means ± S.E. of four experiments, each performed in duplicate.

ble observation has been reported in BALB/c 3T3 preadipose cells [21]. As expected, ouabain-sensitive K^+ influx was not influenced by changes in chloride concentration (data not shown).

As shown in Fig. 3, OR-BS K^+ influx ceases in the absence of external Na^+ or Cl^-. In addition, bumetanide-sensitive influx of both Na^+ and Cl^- was drastically reduced in the absence of external K^+ (data not shown).

Estimation of the stoichiometry of cotransport

To determine the stoichiometry of cotransport, concurrent measurements of OR-BS influx of $^{86}Rb^+$, $^{24}Na^+$ and $^{36}Cl^-$ were made. The results of five experiments revealed a $Na^+/K^+/Cl^-$ stoichiometry of $0.9 \pm 0.2 : 1.0 : 1.8 \pm 0.3$ with the K^+ value set to 1.0. Taken with the results described above, these findings suggest the presence of a $Na^+/K^+/Cl^-$ cotransporter in HT29 cells and that the stoichiometry of the cotransporter is $1Na^+ : 1K^+ : 2Cl^-$.

Lack of volume activation

Changes in cell volume did not affect OR-BS K^+ influx. However, cell swelling did stimulate active K^+ influx as shown in Table II.

ATP dependence of OR-BS K^+ influx

Although $Na^+/K^+/Cl^-$ cotransport is known not to be catalyzed by the hydrolysis of ATP [11], it is nonetheless dependent on ATP content in many cell types [28]. Fig. 4 shows the dependency of HT29 cell cotransport on ATP content, which was manipulated by the treatment of cells with 2-deoxyglucose. With ATP depletion, cotransport was progressively reduced and was not detectable

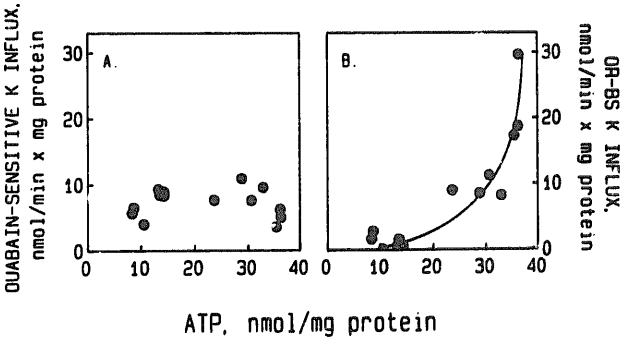

Fig. 4. ATP dependence of K^+ influx. The experimental conditions were similar to those in Fig. 1, except that cells were preincubated with BSS containing 5 mM 2-deoxyglucose, instead of glucose, for various time periods to deplete the cellular ATP content. K^+ influx was then determined as stated in the Materials and Methods in the presence of 2-deoxyglucose. ATP levels were measured from a perchloric acid extract of several dishes just prior to the influx assay. Data points shown are the means of duplicate determinations from three experiments.

TABLE II

K^+ INFLUX IN RESPONSE TO CHANGES IN CELL VOLUME

K^+ influx was measured as stated in the Materials and Methods section, except that cells were preincubated for 10 min in a modified BSS solution. The Na^+ and Cl^- content of BSS were reduced to 85 mM and the osmolarity of the medium was varied by addition of sucrose. Cells remained in this medium during the 5-min influx assay. The values shown are the mean ± S.E. of four experiments, each performed in duplicate.

Influx pathway	Osmolarity (mosM)		
	200	300	400
Ouabain-sensitive K^+ influx	12.3±0.3	2.4±1.8	2.4±0.4
Ouabain-resistant bumetanide-sensitive K^+ influx	18.2±0.3	20.6±1.5	22.9±1.2

near 10 nmol ATP/mg protein (B), a concentration at which K^+ influx by the active transport pathway was still fully operational (A).

Divalent cation dependency

Fig. 5 illustrates the effects on OR-BS K^+ influx of a variety of divalent cations in the presence of the bivalent metal ion ionophore, A23187. While A23187 had no effects on OR-BS K^+ influx, cotransport was decreased approx. 20% in the presence of A23187 and the divalent cations Ca^{2+}, Mg^{2+}, Mn^{2+} and Ba^{2+}. Data are presented as percent of OR-BS K^+ influx in control cells (see below).

Effect of cyclic AMP on OR-BS K^+ influx

Fig. 6 shows the effects of the exogenously added cyclic AMP analogue, 8-bromo-cyclic AMP, and of various agents known to increase intracellular cyclic AMP content in HT29 cells. In addition to the relatively moderate increases in cyclic AMP in response to VIP [16,33], PGE_1 [16] and FSK [15], the sensitization of HT29 cells to FSK by pretreatment with NE results in increases of up to two orders of magnitude [15]. It should be noted that some of these experiments were performed

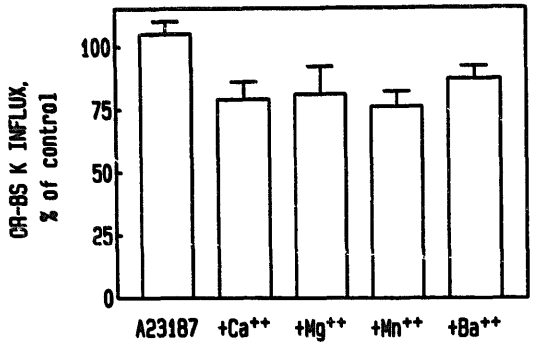

Fig. 5. Effects of divalent cations on OR-BS K^+ influx. Cells were preincubated and OR-BS K^+ influx was measured as stated in the Materials and Methods. A23187 (10 μM) in the absence or presence of the indicated divalent cations (1 mM) was present during both the 10-min BSS pretreatment and the 5-min uptake assay. Each value shown is the mean ± S.E. of duplicate determinations from four experiments. Data is presented as the percent of uptake in cells incubated in the absence of ionophore or divalent cations (see text).

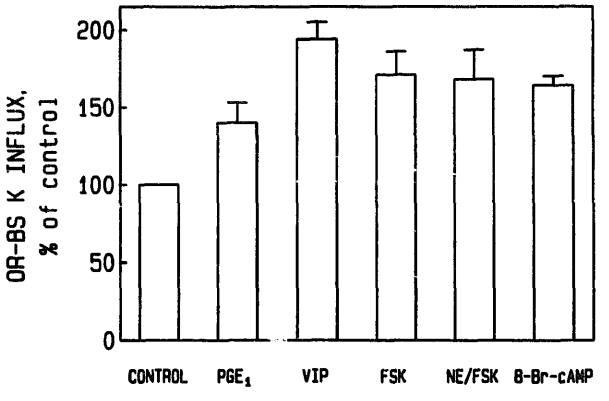

Fig. 6. Effect of 8-bromo-cyclic AMP and agents that increase endogenous cyclic AMP levels on OR-BS K^+ influx. Experimental conditions were similar to those described in Fig. 1. PGE_1 (10 μM) and VIP (10 nM) were added 30 min prior to the initiation of the K^+ influx assay, whereas FSK (10 μM) and 8-bromo-cyclic AMP (1 mM) were added 90 min prior to the influx assay. Sequential treatment with NE and FSK was performed as follows: cells were pretreated with 10 μM NE for 90 min in DMEM-Hepes, washed, and then 10 μM FSK was included in the 10-min BSS preincubation. All agents tested (except NE) were present during the uptake assay. OR-BS K^+ influx was assayed for 5 min. Values shown are the mean ± S.E. for duplicate or quadruplicate determinations and are expressed as the percent of uptake in control cells (see text). The number of experiments for each treatment are shown in parentheses. PGE_1 (4), VIP (9), FSK (5), NE/FSK (8), 8-bromo-cyclic AMP (44).

similarly to those presented in other figures, that is, in 35-mm diameter dishes. Other experiments included in Figs. 5 and 6, however, were performed in 24-well plates, of which each well has 21% of the surface area of a 35-mm dish, resulting in fewer cells (and lower protein) per well. Although cyclic AMP stimulators and analogues consistently stimulated and divalent cations inhibited OR-BS K^+ influx in both types of dish, we have observed an anomalous quantitative difference in uptake between the two types of dish when uptake is expressed as a function of protein concentration. OR-BS K^+ influx in 24-well plates was found to be 4.6 ± 0.5 nmol/min per mg protein ($n = 12$) in control cells, or approx. 25% of that in 35-mm dishes. While we are investigating the basis for this anomaly, the data for Figs. 5 and 6, expressed as a percent of the uptake activity in control cells in each experiment, strongly suggest regulation of $Na^+/K^+/Cl^-$ cotransport in HT29 cells by both divalent cations and cyclic AMP.

Discussion

Since their establishment in culture [6], HT29 cells have been widely used as an epithelial cell model in which to characterize plasma membrane receptors. HT29 cells express α_2-adrenergic [2,34], VIP [1,33] and insulin [7,8] receptors among others. Recently, we have shown an insulin action in HT29 cells in which glycolysis is stimulated by insulin without augmenting glucose transport [8]. Unlike T84 cells, which are also derived from a human colonic adenocarcinoma, relatively little is known about ion-transport characteristics in HT29 cells.

The findings reported herein unequivocally demonstrate the presence of a $Na^+/K^+/Cl^-$ cotransport system in HT29 cells. This is based on (a) a mutual dependency among transported species; (b) an inhibition of ion flux by loop diuretics and (c) an apparent stoichiometry of $1Na^+ : 1K^+ : 2Cl^-$.

In addition, the $Na^+/K^+/Cl^-$ cotransporter is exquisitely sensitive to ATP content, in keeping with similar observations reported in Ehrlich ascites tumor cells [11] and avian red cells [28]. It has been shown that treatment of HT29 cells with VIP [16,33], PGE_1 [16], FSK alone [15] or the sequential treatment of these cells with NE followed by FSK [15] increases the intracellular cyclic AMP content by up to two orders of magnitude. Both exogenously added cyclic AMP analogues, (8-bromo-cyclic AMP (Fig. 6) and dibutyryl-cyclic AMP (data not shown)), as well as increased endogenous cyclic AMP, stimulated the cotransport pathway.

The location of the cotransporter is thought to vary, depending on the cell type. The cotransporter is presumed to be localized in the apical membranes of chloride absorptive epithelial and the basolateral membranes of chloride secretory epithelia [24]. The location of the $Na^+/K^+/Cl^-$ cotransporter in HT29 cells is not known, whereas in T84 cells, Dharmsathaphorn et al. [4] have suggested the basolateral membrane as the likely site for the cotransporter. It was further shown that in T84 cells, VIP, acting through the generation of cyclic AMP, stimulates the $Na^+/K^+/Cl^-$ cotransport pathway, resulting in an intracellular accumulation of Cl^-. Chloride is then thought to diffuse down its chemical potential gradient through chloride channels present in the apical membrane [18]. The basolateral membrane of T84 cells also exhibits Na^+/K^+-ATPase activity and contains cyclic AMP- and Ca^{2+}-dependent K^+ channels [4,18,19]. According to this scheme, Na^+ entering on the basolateral side by way of a $Na^+/K^+/Cl^-$ cotransporter is pumped out of the cells by the Na^+/K^+-ATPase located on the basolateral and interstitial lateral space membranes. Moreover, K^+ entering on the basolateral side through both active transport and cotransport routes leaves the cell through the K^+ channels. The net effect is NaCl secretion.

Of particular interest is the finding that although changes in cell volume, whether shrinking or swelling, do not influence cotransport in HT29 cells, cell swelling apparently does increase active K^+ transport. The so-called regulatory volume increase, which refers to the swelling of hypertonically shrunken cells, is thought to depend upon an activation of a NaCl or $Na^+/K^+/Cl^-$ cotransport system or coupled Na^+/H^+ and Cl^-/HCO_3^- exchange pathways [5,14]. On the other hand, a recovery of cell volume after hypotonic swelling is referred to as a regulatory volume decrease, which relies on KCl cotransport, KCl conductances or

parallel K^+/H^+ and Cl^-/HCO_3^- exchangers [5,14]. In either of the regulatory volume controls, the involvement of the Na^+/K^+ pump is less clear. In this regard, HT29 cells appear to be a valuable model in which to characterize activation of the Na^+/K^+ pump by cell swelling.

Acknowledgements

The authors wish to thank Ms. J. Burnett, M. Katt and P. Hughes for expert assistance, and L. Corkins for skillful typing. We are grateful to Dr. S. O'Grady for providing preprints of a review paper. This work is supported in part by NIH grants DK 33446, DE 07389 and the Edward Mallinckrodt, Jr. Foundation.

References

1 Boissard, C., Marie, J.-C., Hejblum, G., Gespach, C. and Rosselin, G. (1986) Cancer Res. 46, 4406–4413.
2 Bouscarel, B., Murat, J.C. and Paris, H. (1985) Gen. Pharmacol. 16, 641–644.
3 Brock, T.A., Brugnara, C., Canessa, M. and Gimbrone, M.A., Jr. (1986) Am. J. Physiol. 250, C888–C895.
4 Dharmsathaphorn, K., Mandel, K.G., Masui, H. and McRoberts, J.A. (1985) J. Clin. Invest. 75, 462–471.
5 Eveloff, J.L. and Warnock, D.G. (1987) Am. J. Physiol. 252, F1–F10.
6 Fogh, J., Fogh, J.M. and Orfeo, T. (1977) J. Natl. Cancer Inst. 59, 221–226.
7 Forgue-Lafitte, M.E., Horvat, A. and Rosselin, G. (1979) Mol. Cell. Endocrinol. 14, 123–130.
8 Franklin, C.C., Chin, P.C., Turner, J.T. and Kim, H.D. (1988) Biochim. Biophys. Acta 972, 60–68.
9 Garay, R.P. (1982) Biochim. Biophys. Acta 688, 786–792.
10 Geck, P. and Heinz, E. (1986) J. Membr. Biol. 91, 97–105.
11 Geck, P., Pietrzyk, C., Burckhardt, B.-C., Pfeiffer, B. and Heinz, E. (1980) Biochim. Biophys. Acta 600, 432–447.
12 Haas, M., Schmidt, W.F. III and McManus, T.J. (1982) J. Gen. Physiol. 80, 125–147.
13 Hall, A.C. and Ellory, J.C. (1985) J. Membr. Biol. 85, 205–213.
14 Hoffmann, E.K. (1986) Biochim. Biophys. Acta 864, 1–31.
15 Jones, S.B., Toews, M.L., Turner, J.T. and Bylund, D.B. (1987) Proc. Natl. Acad. Sci. USA 84, 1294–1298.
16 Laburthe, M., Rousset, M., Boissard, C., Chevalier, G., Zweibaum, A. and Rosselin, G. (1978) Proc. Natl. Acad. Sci. USA 75, 2772–2775.
17 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
18 Mandel, K.G., Dharmsathaphorn, K. and McRoberts, J.A. (1986) J. Biol. Chem. 261, 704–712.
19 McRoberts, J.A., Beuerlein, G. and Dharmsathaphorn, K. (1985) J. Biol. Chem. 260, 14163–14172.
20 McRoberts, J.A., Erlinger, S., Rindler, M.J. and Saier, M.J., Jr. (1982) J. Biol. Chem. 257, 2260–2266.
21 O'Brien, T.G. and Krzeminski, K. (1983) Proc. Natl. Acad. Sci. USA 80, 4334–4338.
22 O'Donnell, M.E. and Owen, N.E. (1986) Proc. Natl. Acad. Sci. USA 83, 6132–6136.
23 O'Donnell, M.E. and Owen, N.E. (1986) J. Biol. Chem. 261, 15461–15466.
24 O'Grady, S.M., Palfrey, H.C. and Field, M. (1987) Am. J. Physiol. 253, C177–C192.
25 O'Grady, S.M., Field, M., Nash, N.T. and Rao, M.C. (1985) Am. J. Physiol. 249, C531–C534.
26 Owen, N.E. and Prastein, M.L. (1985) J. Biol. Chem. 260, 1445–1451.
27 Owen, N.E. (1984) Biochem. Biophys. Res. Commun. 125, 500–508.
28 Palfrey, H.C. and Rao, M.C. (1983) J. Exp. Biol. 106, 43–54.
29 Palfrey, H.C. and Greengard, P. (1981) Ann. N.Y. Acad. Sci. 372, 291–308.
30 Palfrey, H.C., Alper, S.L. and Greengard, P. (1980) J. Exp. Biol. 89, 103–115.
31 Paris, S. and Pouysségur, J. (1986) J. Biol. Chem. 261, 6177–6183.
32 Rindler, M.J., McRoberts, J.A. and Saier, M.H., Jr. (1982) J. Biol. Chem. 257, 2254–2259.
33 Turner, J.T., Jones, S.B. and Bylund, D.B. (1986) Peptides 7, 849–854.
34 Turner, J.T., Ray-Prenger, C. and Bylund, D.B. (1985) Mol. Pharmacol. 28, 422–430.
35 Ueberschar, S. and Bakker-Grunwald, T. (1983) Biochim. Biophys. Acta 731, 243–250.