J. Membrane Biol. 130, 83–90 (1992)

The Journal of
Membrane Biology

Activation of Amino Acid Diffusion by a Volume Increase in Cultured Kidney (MDCK) Cells

G. Roy and C. Malo
Groupe de Recherche en Transport Membranaire, Département de Physiologie, Université de Montréal, Montréal, Québec, Canada H3C 3J7

Summary. When MDCK cells are cultured in MEM, they maintain a high concentration of three amino acids: glutamate (25 mM), taurine (19 mM) and glycine (9 mM). With incubation of the cells in hypotonic media, the contents of these amino acids measured by HPLC are reduced in different time courses: taurine decreases most rapidly, followed by glutamate and glycine. All these losses are Na^+ independent. To determine the transport mechanism activated by the hypotonic media, increasing external concentrations reaching 60 mM for nine different amino acids in Na^+-free media were tested separately. For the five neutral (zwitterionic) amino acids, taurine, glycine, alanine, phenylalanine and tryptophan, cell contents increased linearly with external concentrations in hypotonic media, whereas in isotonic media only a slight rise was observed. The two anionic amino acids, glutamate and aspartate, were also increased linearly with their external concentrations in hypotonic media, but the changes were lower than those found for neutral amino acids. The presence of a negative membrane potential was responsible for this behavior since, using a K^+ hypotonic medium which clamps the potential to zero, the glutamate content was found to increase linearly with an amplitude similar to the one observed for neutral amino acid. When external concentrations of two cationic amino acids, arginine and lysine, were increased in hypotonic media, only a small change, similar to that in isotonic media, was observed. These results indicate that a diffusion process for neutral and anionic amino acids is activated by a volume increase and it is suggested that an anion channel is involved.

Key Words amino acid · volume regulation · MDCK cells · hyposmolarity · kidney cells

Introduction

An impressive number of studies have been performed recently on the activation of K^+ and Cl^- membrane transport after a cell volume increase induced by hypotonic media [5, *for review*]. The K^+ and Cl^- losses occurring in those conditions could explain most of the regulatory volume decrease (RVD) observed, if the cells belong to the class of low osmolarity organisms (300 mOsm). Cells of marine species with high osmolarity (1000 mOsm) contain a large proportion of organic osmolytes which are also lost during RVD [7]. Mammalian cells contain an important concentration of organic osmolytes, particularly amino acids, as found in Ehrlich ascites tumor cells [9] and in MDCK cells [12, 14]. The total concentration of amino acids in these cases is between 60–69 mM and therefore represents an important pool of organic osmolytes. In both these cell types, a large fraction of their amino acids is lost during RVD. Sixteen free amino acids in MDCK cells [4] are reduced during RVD. Very few mammalian cells have been studied with respect to their organic osmolyte concentrations and their losses during RVD. Kidney cells, especially those of the collecting duct, are frequently exposed to large variations of external osmolarity. It is known that renal medullary cells change their organic osmolyte concentrations during their adaptation to media of varying osmolarity [6]. It is therefore important to determine organic osmolyte losses in kidney cells during volume increases activating membrane transport processes. A loss of organic osmolytes in hypotonic conditions could have implications in the metabolic activities of cells, as demonstrated in the liver [8].

Until now, the amino acid transport mechanism activated by volume regulation has not been clearly identified. A passive leak may be involved in Ehrlich ascites tumor cells [9]. In MDCK cells, quinidine and stilbene derivative inhibitor of Cl^- channels (DIDS) suppress taurine release during RVD, whereas other inhibitors of Cl^- and K^+ channels are ineffective [14]. Taurine release in astrocytes incubated in hypotonic media is mediated by diffusion [13]. In the present study, neutral (glycine, taurine, alanine, phenylalanine and tryptophan), anionic (glutamate and aspartate) and cationic (lysine and arginine) amino acids were measured in cells incubated in

hypotonic media to determine the type of amino acid transport system activated during RVD in cultured MDCK cells.

Materials and Methods

Cultures

The MDCK line was obtained from the American Type Culture Collection at 58 serial passages. The cells were seeded at medium density and grown until confluence in Minimal Essential Medium (MEM) (GIBCO, Grand Island, NY) in plastic bottles (Falcon Labware, Becton Dickinson Canada, Toronto, Ont.). Experiments were performed with confluent monolayers ($3.5–4.0 \times 10^5$ cells/cm^2). The medium contained gentamicin (10 mg/liter) and 10% fetal bovine serum (GIBCO). It was changed every two days. Cultures were maintained at 37°C in a humid air atmosphere and the Falcon bottles were kept closed. Subculture was performed by detaching the cells from the bottles, using a trypsin (0.05%)-EDTA (0.02%) solution.

Solutions

MEM was buffered at pH 7.3 with (in mM): 25 HEPES, 10 NaOH and 6 $NaHCO_3$. Earle's medium contained (in mM): 121 NaCl, 5.4 KCl, 25 HEPES, 10 NaOH, 6 $NaHCO_3$, 5.6 glucose, 0.8 $MgSO_4$, 1.8 $CaCl_2$, 1.0 NaH_2PO_4. Na^+-free medium was prepared by replacing all Na^+ by *n*-methyl-D-glucamine ($NMDG^+$). Amino acid Na^+-free media were produced by reducing the NMDG-Cl^- concentration. Negatively charged amino acids (glutamate and aspartate) had a decreased Cl^- concentration and positively charged ones (arginine and lysine) a diminished $NMDG^+$ concentration. Hypotonic media were obtained by adding water to isotonic media. All the external amino acid concentrations given in the figures are those of the isotonic media. The osmolality of each medium was measured with an osmometer (Advanced Instruments, Needham Heights, MA). The Na^+ content was measured with a flame photometer (Instrumentation Laboratory, Fisher Scientific, Montréal, Canada) as previously described [12].

Determination of Amino Acid Content

The amount of amino acids in intra- and extracellular media was determined by reverse phase high performance liquid chromatography (HPLC) after pre-column derivatization with *o*-phthaldehyde (OPA) reagent. Elution was performed by using a discontinuous gradient of solvent A (tetrahydrofuran-methanol-sodium acetate buffer, 0.1 M, pH 6.8; 5 : 95 : 900) and B (methanol) [11]. Standard amino acid solutions (25 μM) were prepared in bidistilled water. Samples of cell hydrolysates and incubation media were boiled for 10 min in bidistilled water containing 25 μM α-amino-β-guadinino propionic acid as internal standard and were centrifuged for 15 min at 100,000 × *g*. The supernatants were filtered on 0.22 μm Millipore GS (Mississauga, Ont.) filters.

The fluoraldehyde (FA) reagent was prepared weekly by mixing 50 mg OPA dissolved in 1.25 ml methanol, 50 μl β-mercaptoethanol, 11.2 ml sodium borate buffer, 0.2 M, pH 9.5 and 50 μl Brij 35. The derivatization procedure was as follows: 50 μl of standard solution or sample and 50 μl of external standard (α-amino caprylic acid, 25 μM) were mixed with 50 μl of FA. The reaction was stopped after 1 min by adding 72 μl of 1% acetic acid which gives a final pH of 6.8. The volume was completed to 2 or 4 ml by adding 0.1 M sodium acetate buffer, pH 6.8, and 20 μl of the mixture was subjected to analysis.

The HPLC apparatus was a two-pump system (Beckman Instruments, Toronto, Ont.) coupled to a fluorometer equipped with filters for excitation at 305–395 nm and emission at 420–650 nm. Separation was performed on a reverse-phase Ultrasphere ODS column (4.6 nm I.D.; particle size 5 μm; length 150 mm) fitted with a guard column of a shorter length (45 mm) but with the same physical characteristics (Beckman Instruments).

Amino acids were analyzed quantitatively by measuring the relative height of an individual amino acid over the height of the external standard of known concentration and by comparing these ratios with those measured on standard chromatographic amino acid profiles.

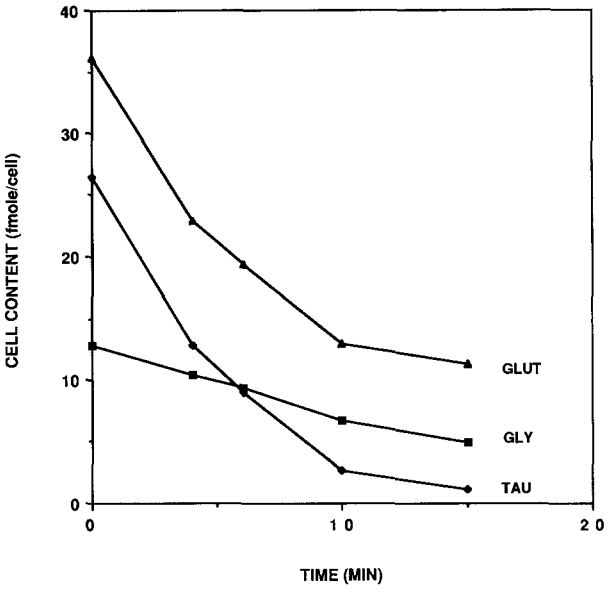

Fig. 1. Amino acid contents in MDCK cells in hypotonic medium. Time course of glutamate, taurine and glycine contents measured from a HPLC profile analysis after incubation in a 125-mOsm Earle's medium. The data points were obtained from a single typical experiment.

Results

Reduction of Amino Acid Content in Hypotonic Media

From the HPLC profiles, we observed that three amino acids were much more concentrated than all the others, that is: glutamate > taurine > glycine. These three amino acids were measured as a function of time during incubation of the cells in the hypotonic medium (125 mOsm). As shown in Fig. 1, the rate of release was different for each amino acid

Table 1. Effect of hypotonic medium on amino acid content

Amino acid	Intracellular (fmol/cell)		Extracellular (fmol/cell)	
	290 mOsm	125 mOsm	290 mOsm	125 mOsm
A) Normal Na^+ medium				
Glutamate	38 ± 6	12 ± 2	0	28 ± 4
Taurine	28 ± 3	4.5 ± 1.5	0	26 ± 4
Glycine	13 ± 2	6.0 ± 1.1	0	6.5 ± 1.2
B) Na^+-free medium				
Glutamate	35 ± 6	11 ± 2	0	25 ± 4
Taurine	26 ± 3	5.2 ± 1.5	0	23 ± 4
Glycine	12 ± 2	6.7 ± 1.1	0	6.1 ± 1.2

Cells were incubated for 10 min in either isotonic (290 mOsm) or hypotonic (125 mOsm) media. In B, cells were preincubated for 15 min in Na^+-free isotonic medium. The results represent average ± SD from three experiments performed at room temperature.

studied, the taurine loss being the fastest, and glycine the slowest. If the hypotonic medium was maintained for 30 min or 1 hr, amino acid contents remained at their 15 min values (*data not shown*). To verify if these reductions were related to an increase in their membrane transport or to a change in metabolic activity, the extracellular contents of these three amino acids were measured with their cellular levels. As shown in Table 1(A), there was no measurable amount of amino acid in the isotonic medium during 10 min, whereas the amino acids measured in the hypotonic medium during the same period corresponded quite closely to their cell losses, indicating that there was no appreciable change in metabolic activity during those 10 min. From the intracellular contents of these three amino acids in isotonic media [Table 1(A)] and using a cell water volume of 1.5 pl as measured previously [12] in the same conditions, their normal cellular concentrations were calculated: glutamate concentration was 25 mM, taurine 19 mM and glycine 9 mM. Based on our previous estimates [12] of total amino acid concentration (66 mM), these three amino acids accounted for 80% of the total intracellular amino acid concentration.

Influence of Na^+-Dependent Transport on Amino Acid Efflux

The large efflux of these three amino acids induced by cell swelling in a hypotonic medium could be either Na^+ dependent or independent. These possibilities were tested by incubating the cells in isotonic medium without Na^+ for 15 min to deplete intracellular Na^+. The normal Na^+ content of 0.03 ± 0.005 pmol/cell ($n = 5$) was reduced to zero after 15 min of incubation in Na^+-free medium. Afterwards, hypotonic medium without Na^+ was introduced for 10 min, and the intra- and extracellular contents of glutamate, taurine and glycine were measured. As shown in Table 1(B), the results were the same within experimental error as those obtained in Na^+-containing Earle's media [Table 1(A)], indicating that the large amino acid efflux induced by a volume increase is not related to a Na^+-dependent transport process.

Effects of External Amino Acids on their Cellular Contents in Hypotonic Media

To determine the amino acid transport mechanism activated by a volume increase, escalating external concentrations of amino acids, reaching 60 mM, were introduced separately in the isotonic and hypotonic Na^+-free media, and cell content was measured after a 10-min incubation period. Intracellular amino acid content should reflect the net flux which should be modified by increasing concentrations of amino acids in the external medium. In this study, nine different amino acids were evaluated: five neutral (glycine, taurine, alanine, phenylalanine and tryptophan), two anionic (glutamate and aspartate) and two cationic amino acids (lysine and arginine).

Neutral Amino Acids

As shown in Fig. 2*A*, *B* and *C*, 1 mM taurine, glycine or alanine in the external hypotonic Na^+-free medium did not change their cell content after 10 min. When the external concentrations were raised from 10 to 60 mM in hypotonic medium, the cellular content of these amino acids increased linearly. The intracellular amino acid contents reached at 60 mM

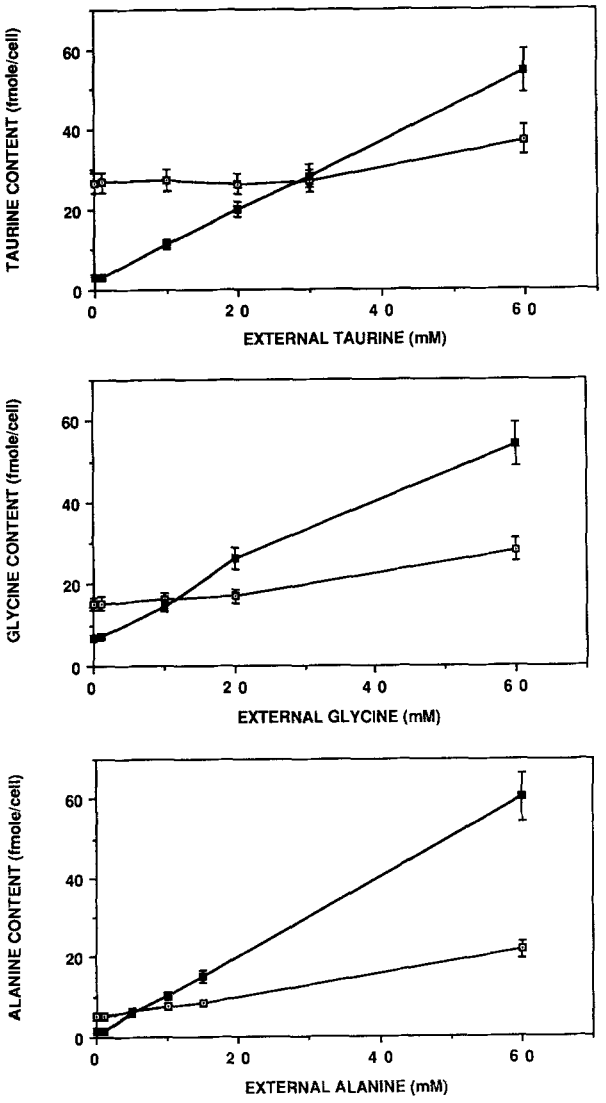

Fig. 2. Effect of external amino acid concentrations on MDCK cell amino acid contents after 10 min of incubation in isotonic or hypotonic Na^+-free media. The data values were obtained from a HPLC profile analysis, giving the taurine (*A*), glycine (*B*) and alanine (*C*) contents in hypotonic (■) and isotonic (□) media. Each data point represents the mean ± SD from three separate experiments.

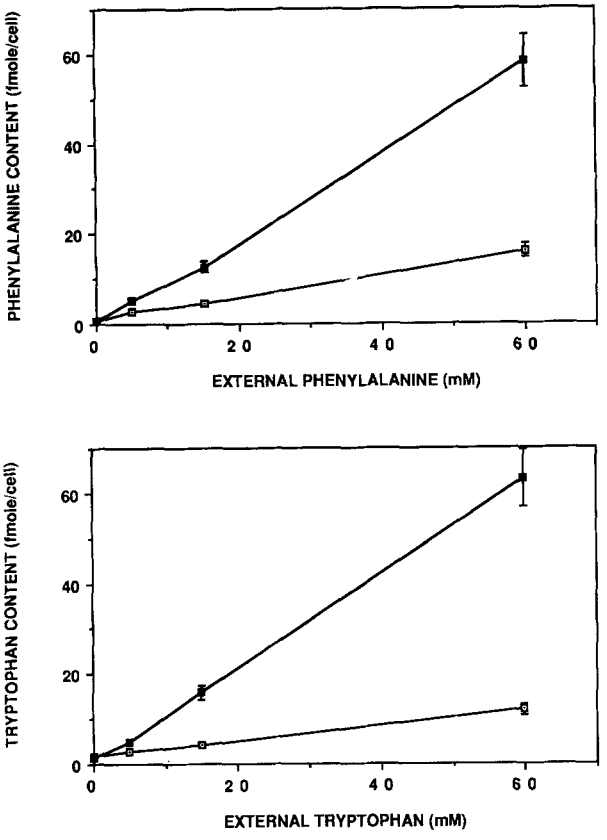

Fig. 3. Effect of external amino acid concentrations on MDCK cell amino acid contents after 10 min of incubation in isotonic or hypotonic Na^+-free media. The data points were obtained from a HPLC profile analysis, giving phenylalanine (*A*) and tryptophan (*B*) contents in hypotonic (■) and isotonic (□) media. Each data point represents the mean ± SD from three separate experiments.

external concentration were similar and represent 2 to 10 times their normal content. As shown in Fig. 2, the external amino acid concentration preventing a reduction of the normal cell content in hypotonic medium was different for each amino acid tested: for taurine, 30 mM was required, while 10 and 5 mM were sufficient for glycine and alanine, respectively. In isotonic medium, significant changes in cell content were observed only when 60 mM amino acid concentration was introduced in the external medium. Obviously, these changes were always much less than those observed in the hypotonic media.

To determine if the Na^+-dependent taurine influx could reduce the intracellular loss of taurine during 10 min of incubation in hypotonic medium, Earle's medium with Na^+ and 1 mM taurine was tested; this concentration has been shown to saturate Na^+-dependent taurine transport in MDCK cells [10]. Under these conditions, the data obtained were similar (within the same experimental error) to those presented in Fig. 2*A*, meaning that the Na^+-dependent taurine influx was not large enough to balance the large efflux produced by a volume increase.

Two large neutral amino acids were also studied, namely, phenylalanine and tryptophan. Under control conditions, the phenylalanine content was (in fmol/cell) 0.88 ± 0.09 (n = 3) and that of tryptophan was 1.02 ± 0.15 (n = 3). In hypotonic media, these contents were reduced to 0.56 ± 0.06 (n = 3) and 0.68 ± 0.07 (n = 3) for phenylalanine and tryptophan, respectively. These cellular levels were very

low and exhibited only a small 35% reduction in the hypotonic medium. When the external concentration of phenylalanine or tryptophan was increased to 5, 15 and 60 mM in hypotonic media, a large and linear increase was observed (Fig. 3*A* and *B*). The intracellular amino acid contents reached were similar to those measured for taurine, glycine and alanine under similar experimental conditions (Fig. 2). For both phenylalanine and tryptophan, a small increase in their cell contents was observed in isotonic medium.

After increasing the cellular levels of these two amino acids with 15 mM external concentration in hypotonic media, we determined to what values they would be reduced in hypotonic media without amino acids. Under these conditions, the phenylalanine and tryptophan contents were reduced respectively to 4.8 ± 0.5 (n = 2) and 6.1 ± 0.9 fmol/cell (n = 2), corresponding to a 60% loss within 10 min, a much larger release than that observed in cells with normal amino acid contents.

Anionic Amino Acids

Similar experiments were performed with aspartate and glutamate (Fig. 4). Increasing the external concentration of aspartate from 1 to 60 mM in the hypotonic medium induced a linear rise in its cellular level (Fig. 4*A*); 20 to 30 mM was sufficient to prevent a cellular loss and 60 mM produced an increase of aspartate content. Increases also occurred in the isotonic medium, intracellular contents being similar for both iso- and hypotonic media when 60 mM aspartate was introduced in the external medium.

The normal intracellular glutamate level was fairly high (Fig. 4*B*) compared to aspartate (Fig. 4*A*), and much greater external concentrations were necessary to prevent cellular losses in hypotonic media. Even when 40 mM glutamate was introduced in the hypotonic medium, no change was observed in the glutamate loss. A reduction of glutamate loss was noted only when 80 mM was used, and 120 mM was required to balance the glutamate efflux. Again, the intracellular levels of glutamate appeared to be linearly related to its external concentrations. However, under such experimental conditions, a large amount of external Cl^- was replaced by glutamate and significant increase of glutamate in cells in the isotonic medium was found (Fig. 4*B*). To determine if the Na^+-dependent glutamate influx could compensate for the loss of glutamate during 10 min of hypotonic incubation, Earle's medium with Na^+ and 1 mM glutamate was used. The glutamate loss was similar to that observed under Na^+-free conditions (Fig. 4*B*), again indicating that Na^+-dependent

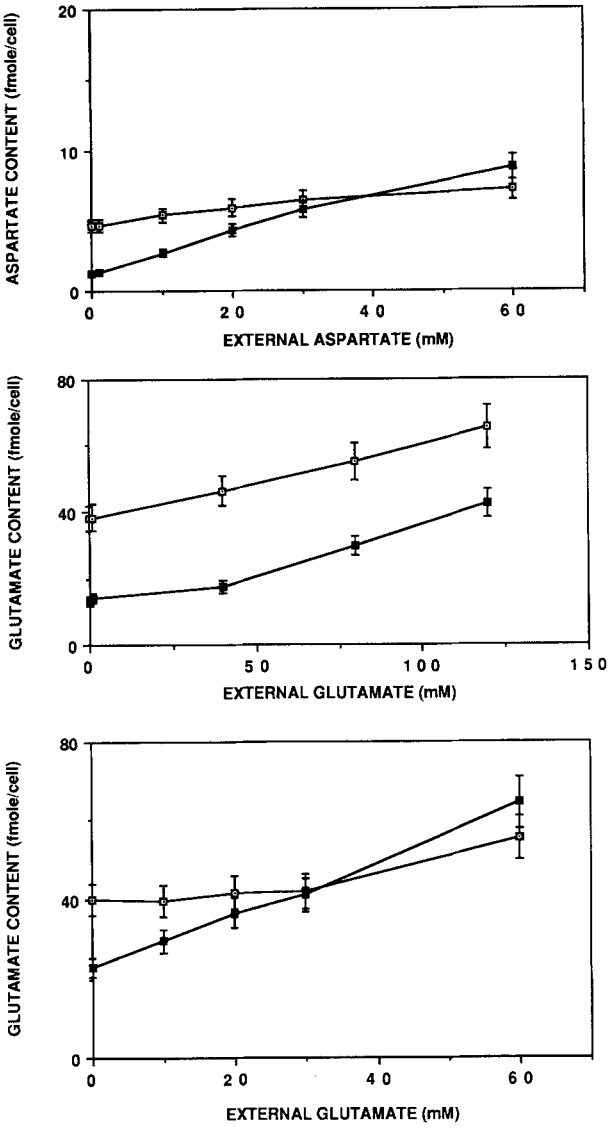

Fig. 4. Effect of external amino acid concentrations on MDCK cell amino acid contents after 10 min of incubation in isotonic (□) or hypotonic (■) Na^+-free media. The data points were obtained from a HPLC profile analysis, giving the aspartate (*A*) and glutamate (*B* and *C*) contents. Glutamate content in (*C*) was determined with NMDG-Cl^- in Earle's medium replaced by KCl (50%) and K-gluconate (50%). Each data point represents the mean ± SD from three separate experiments.

transport was not large enough to prevent the rapid efflux occurring during hypotonic swelling.

The aspartate and glutamate cell contents (Fig. 4*A* and *B*) in 60 mM amino acid in hypotonic media were much lower than those measured for the neutral amino acids (Figs. 2 and 3). Also, the 120 mM external concentration required to balance the loss of glutamate in the hypotonic medium was much larger than the cell concentration (25 mM) calculated from its cell contents. These effects could have been

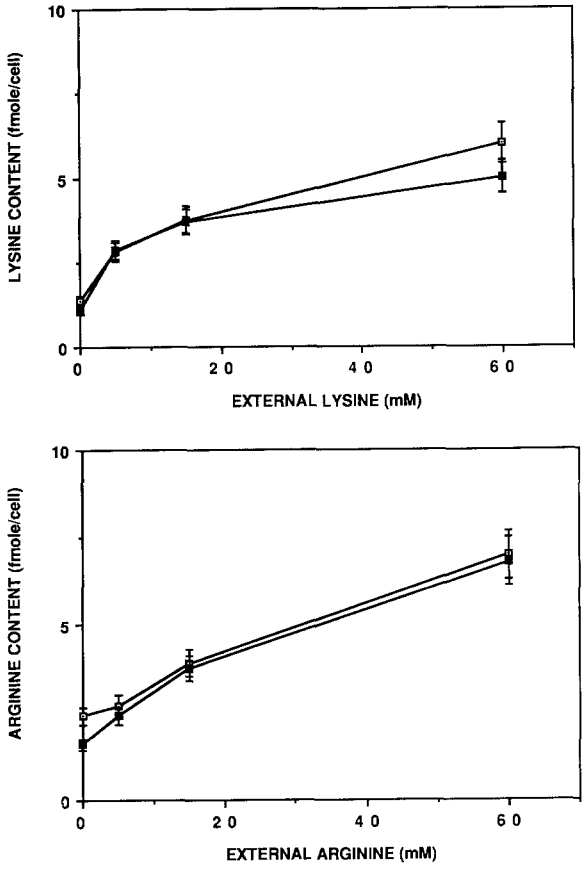

Fig. 5. Effect of external amino acid concentrations on MDCK cell amino acid contents after 10 min of incubation in isotonic (□) or hypotonic (■) Na^+-free media. The data values were obtained from a HPLC profile analysis, giving arginine (*A*) and lysine (*B*) contents. Data points represent mean ± SD from three separate experiments.

induced by negative membrane potential which should decrease the influx of negatively charged molecules. Under such conditions, a much larger external concentration would be required to produce an influx equal to the efflux. To test this possibility, experiments were performed under zero membrane potential, a condition achieved by replacing external Na^+ by K^+. To prevent slow swelling, which normally occurs in these conditions, half of the Cl^- was replaced by gluconate. In such a medium, cells are depolarized and retain stable K^+ and Cl^- contents [12]. As shown in Fig. 4*C*, there was a loss of glutamate in hypotonic medium. A linear increase of the glutamate contents was observed as the external concentrations were raised from 10 to 60 mM, and intracellular levels reached were similar to those obtained for neutral amino acids (Figs. 2 and 3). External glutamate concentrations around 30 mM were sufficient to balance the efflux. In isotonic medium, an increase in glutamate content was observed only when 60 mM glutamate was introduced in the external medium.

Table 2. Relative release of amino acids in hypotonic medium

Amino acid	% Release	Mol wt	Net charge (pH 7)
Taurine	84	125	0
Glutamate	69	147	−1
Aspartate	67	133	−1
Alanine	62	90	0
Phenylalanine	61[a]	165	0
Tryptophan	60[a]	204	0
Glycine	54	75	0
Arginine	30	174	+1
Lysine	31	146	+1

Cells were incubated for 10 min in either isotonic (290 mOsm) or hypotonic (125 mOsm) Na^+-free media. These results were obtained from three separate experiments. Percent release was calculated from the ratio of the contents in hypotonic and isotonic media.

[a] The values for phenylalanine and tryptophan were based on release after loading in hypotonic media.

Cationic Amino Acids

Finally, two cationic amino acids, arginine and lysine, were studied. As shown in Fig. 5*A* and *B*, their normal levels were low and both were reduced by 20–30% in hypotonic medium. Contrary to neutral and negatively charged amino acids, the intracellular contents of both arginine and lysine were increased to the same extent in either isotonic or hypotonic media when their external concentrations were raised.

The relative release of amino acids under hypotonic conditions is summarized in Table 2. The percent releases given for phenylalanine and tryptophan are those obtained after the cells had been loaded to higher levels with 15 mM external concentration. It appears that these relative releases were not correlated with molecular weight of the individual amino acids studied. The percent release is larger for anionic and neutral amino acid than for cationic amino acids, suggesting some selection based on the net charge of the molecule.

Discussion

The most concentrated amino acids in our MDCK cells were glutamate (25 mM), taurine (19 mM) and glycine (9 mM). These values are slightly different from those reported by Sanchez-Olea et al. [14], who have identified glycine and glutamate as the most

concentrated species followed by alanine and taurine. This disparity is most probably due to the different culture media used. In our case, MEM does not contain any of these amino acids, whereas glycine and serine are present in Dulbecco's modified Eagle medium [14]. It would seem that the presence of glycine and serine in culture medium has an important influence on glutamate, taurine and alanine metabolism which are absent from our culture media and must therefore be produced by cell metabolism. Moreover, the membrane must be quite impermeable to them under normal conditions, although very efficient re-uptake through Na^+-dependent cotransport could play an important role [10].

The cytoplasmic concentration of each amino acid can be calculated from the cell content and the cell volume, as shown above for glutamate, taurine and glycine. For alanine and aspartate, a cellular concentration of about 5 mM can be estimated from their normal content (Figs. 2 and 3) and using a cell volume of 1.5 pl/cell, as above. The external concentrations required to prevent a loss of glutamate, taurine, glycine, aspartate and alanine in hypotonic media constitute another method for evaluating their cytoplasmic levels. In fact, when the external concentration is sufficient to produce an influx equal to the efflux, no change in intracellular levels should be observed in the hypotonic medium if the process is Na^+-independent. From these measurements, the internal concentrations would be about 27 mM for taurine (Fig. 2*A*), 10 mM for glycine (Fig. 2*B*) and 5 mM for alanine (Fig. 2*C*). These values for glycine and alanine were close to those calculated from their content and cell volume, while that for taurine was a little smaller. Applying the same method to evaluate glutamate and aspartate internal concentrations would give 120 and 30 mM, respectively. These values are five to six times larger than those obtained from the content measurements. As shown in Fig. 4*C*, the external glutamate concentration required to prevent any cellular loss was reduced to 30 mM under zero membrane potential conditions and the glutamate content changes in hypotonic conditions were similar to those observed for neutral amino acids, indicating that the net flux of acidic amino acids is largely influenced by a negative inside membrane potential.

Our main interest in this study was to determine the type of transport mechanism activated in hypotonic conditions. It is clear that the decrease in the amino acid content is entirely due to a loss through the plasma membrane and not to a change in metabolic activity. Moreover, the transport mechanism is not Na^+ dependent, since complete Na^+ removal from internal and external media does not alter the efflux. The amino acid transport activated by hypotonic conditions could thus occur through Na^+-independent carrier or through passive diffusion. Both types of transport are present in epithelial membranes, as discussed by Stevens et al. [17]. When a carrier is involved, a saturation of the flux is usually observed as the substrate concentration is increased and a K_m value is obtained when the substrate flux has reached half of the saturation level. Two types of flux are frequently observed: one saturating superposed on another one increasing linearly with substrate concentration. Such results are usually interpreted as evidence for the presence of a carrier and of passive diffusion working in parallel. The most ubiquitous Na^+-independent carrier for neutral amino acid is system L which has a K_m value of 0.7 mM for alanine and 0.3 mM for phenylalanine in intestinal basolateral membranes [17]. System L has also been identified in MDCK cells in isotonic conditions [3]. However, since the substrate concentrations reached in our experiments were much larger than the ones expected to produce a saturation of this carrier, Na^+-independent systems, such as system L, seem not to be the major pathway for amino acid losses observed in MDCK cells in hypotonic conditions. In our experiments, the net flux of each of the five neutral amino acids and of glutamate under zero membrane potential had a similar linear dependence on the external concentration, and no saturation appears even when the external concentration has reached 60 mM. If another hypothetical carrier has to be considered, its K_m would be very large (over 60 mM) and outside the range for any known amino acid carrier.

Thus, simple diffusion appears to be the most likely mechanism responsible for the amino acid losses in hypotonic medium. Passive diffusion plays an important role particularly in the exodus of amino acids across the basolateral membrane [17]. A diffusion mechanism was also proposed recently to account for the losses of taurine in astrocytes incubated in hypotonic media [13]. Although passive diffusion has been shown in many experiments measuring amino acid fluxes across membranes [15], the mechanism involved is not well understood. It was shown that the measured permeabilities are too large to be explained by a diffusion across the lipid bilayer [4]. In this context, it is interesting to compare the relative losses of each of the nine amino acids under hypotonic conditions, as shown in Table 2. Taurine has the highest percent release, followed by a group of six amino acids, namely, glutamate, aspartate, alanine, phenylalanine, tryptophan and glycine, which exhibit similar percent release values. The difference of percent release between taurine and glycine is similar to the permeability difference reported for Ehrlich tumor cells [9]. It should be men-

tioned that for phenylalanine and tryptophan, the percent release values were calculated on the basis of their losses after incubation in 15 mM external concentration. These conditions raised their intracellular levels to the range of the more concentrated amino acids, and their percent release was further increased. Finally, the cationic amino acids, arginine and lysine, have the lowest percent release. From Table 2, it can be seen that no correlation exists between the percent losses and the molecular weight of the individual amino acids tested. Glycine, which has the lowest molecular weight, is in fact the least permeable among the neutral and anionic amino acids. The fact that the cationic amino acids have a very low relative permeability indicates that a selection mechanism based on net charge is involved.

The large and rapid diffusion of neutral and anionic amino acids activated by cell swelling could be compatible with channel properties. Such a channel would be permeable to anionic and neutral amino acids, but not to cationic ones. Recently, it was shown in cultured renal papillary cells that a large increase in sorbitol efflux appeared when the cells swelled following a reduction of osmolarity [6, 16]. The fluxes were linearly dependent on sorbitol concentrations [16] and it was concluded that a channel could be responsible for these fluxes and those of related molecules like mannitol and myo-inositol. An important aspect of those sorbitol fluxes was that among a large number of inhibitors tested, only quinine was very efficient [16]. It should be recalled that the total amino acid losses we have previously measured during volume regulation in MDCK cells were also inhibited by quinine [12]. In addition, we have recently demonstrated [1, 2], through single channel measurements, that quinine is a very potent inhibitor of anion channels. It was also found [2, 12] that this anion channel activated by volume regulation was not very selective among anions. All these observations suggest the possibility that an unselective channel permeable to neutral and anionic molecules is activated by cell swelling. If a channel is mediating the passive diffusion component of amino acid transport, it would provide an explanation for the large values of the diffusion permeability frequently observed in amino acid transport experiments and which have remained unexplained [15]. Therefore, the hypothesis of amino acid diffusion through a channel appears interesting and deserves further studies through single channel experiments.

The authors wish to express their gratitude to Mrs. Julie Verner for her expert assistance. This research was supported in part by grants from the National Sciences and Engineering Research Council of Canada, the Medical Research Council of Canada, Fonds de la Recherche en Santé du Québec and Fonds pour la Formation de Chercheurs et l'Aide à la Recherche du Québec.

References

1. Banderali, U., Roy, G. 1991. Ionic conductances activated during volume regulation in MDCK cells: a patch clamp study. *Biophys. J.* **59**, 254*a*
2. Banderali, U., Roy, G. 1992. Activation of K^+ and Cl^- channels in MDCK cells during volume regulation in hypotonic media. *J. Membrane Biol.* **126**:219–234
3. Boerner, P., Evans-Layng, M., Sang, H., Saier, M.H. Jr. 1986. Polarity of neutral amino acid transport and characterization of a broad specificity transport activity in a kidney epithelial cell line, MDCK. *J. Biol. Chem.* **261**:13957–13962
4. Brunner, J., Graham, D.E., Hauser, H., Semenza, G. 1980. Ion and sugar permeabilities of lecithin bilayers: comparison of curved and planar bilayers. *J. Membrane Biol.* **57**:133–141
5. Chamberlain, M.E., Strange, K. 1989. Anisosmotic cell volume regulation: a comparative review. *Am. J. Physiol.* **257**:C159–C173
6. Garcia-Perez, A., Burg, M.B. 1991. Role of organic osmolytes in adaptation of renal cells to high osmolality. *J. Membrane Biol.* **119**:1–13.
7. Gilles, R. 1979. Intercellular organic osmotic effectors. *In:* Mechanism of Osmoregulation in Animals: Maintenance of Cell Volume. R. Gilles, editor. pp. 111–154. New York, John Wiley & Sons.
8. Haussinger, D., Lang, F. 1989. Exposure of perfused liver to hypotonic conditions modifies cellular nitrogen metabolism. *J. Cell. Biochem.* **43**:355–361
9. Hoffmann, E.K., Lambert, I.H. 1983. Amino acid transport and cell volume regulation in Ehrlich ascites tumor cells. *J. Physiol.* **338**:613–625
10. Jones, D.P., Miller, L.A., Russell, W.C. 1990. Adaptive regulation of taurine transport in two continuous renal epithelial cell lines. *Kidney Int.* **38**:219–226
11. Jones, B.N., Gilligan, J.P. 1983. *o*-Phthaldialdehyde precolumn derivatization and reverse-phase high performance liquid chromatography of polypeptide hydrolysates and physiological fluids. *J. Chromatogr.* **266**:471–482
12. 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
13. Sanchez-Olea, R., Moran, J. Shousboe, A., Pasantes-Morales, H. 1991. Hyposmolarity-activated fluxes of taurine in astrocytes are mediated by diffusion. *Neurosci. Lett.* **130**:233–236
14. Sanchez-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
15. Semenza, G., Corcelli, A. 1986. The absorption of sugars and amino acids across the small intestine. *In:* Molecular and Cellular Basis of Digestion. P. Denuelle, editor. pp. 381–412. Amsterdam, Elsevier
16. Siebenz, A.W., Spring, K.R. 1989. A novel sorbitol transport mechanism in cultured renal papillary epithelial cells. *Am. J. Physiol.* **257**:F937–F946
17. Stevens, B.R., Kaunitz, J.D., Wright, E.M. 1984. Intestinal transport of amino acids and sugars: Advances using membrane vesicles. *Annu. Rev. Physiol.* **46**:417–433

Received 19 March 1992; revised 5 May 1992