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Osmolarity-Sensitive Release of Free Amino Acids from Cultured Kidney Cells (MDCK)

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Summary. The amino acid pool of MDCK cells was essentially constituted by alanine, glycine, glutamic acid, serine, taurine, lysine, β-alanine and glutamine. Upon reductions in osmolarity, free amino acids were rapidly mobilized. In 50% hyposmotic solutions, the intracellular content of free amino acids decreased from 69 to 25 mM. Glutamic acid, taurine and β-alanine were the most sensitive to hyposmolarity, followed by glycine, alanine and serine, whereas isoleucine, phenylalanine and valine were only weakly reactive. The properties of this osmolarity-sensitive release of amino acids were examined using ^{3}H-taurine. Decreasing osmolarity to 85, 75 or 50% increased taurine efflux from 0.6% per min to 1.6, 3.5 and 5.06 per min, respectively. The time course of ^{3}H-taurine release closely follows that of the regulatory volume decrease in MDCK cells. Taurine release was unaffected by removal of Na^+, Cl^- or Ca^{2+}, or by treating cells with colchicine or cytochalasin. It was temperature dependent and decreased at low pH. Taurine release was unaffected by bumetanide (an inhibitor of the $Na^+/K^+/2Cl^-$ carrier); it was inhibited 16 and 67 by TEA and quinidine (inhibitors of K^+ conductances), unaffected by gadolinium or diphenylamine-2-carboxylate (inhibitors of Cl^- channels) and inhibited 50% by DIDS. The inhibitory effects of DIDS and quinidine were additive. Quinidine but not DIDS inhibited taurine uptake by MDCK cells.

Key Words MDCK cells · amino acid release · taurine · volume regulation · quinidine · DIDS

Introduction

Cultured cells from the MDCK line are able to regulate cell volume in anisotonic conditions. MDCK cells exposed to a medium of reduced osmolarity respond initially by marked swelling followed by a slower phase of volume restoration, which occurs despite the persistence of the hyposmotic condition. This process of volume adjustment is accomplished by the extrusion of osmotically active solutes, mainly K^+ and Cl^- (Simmons, 1984; Roy & Sauvé, 1987; Völkl, Paulmichl & Lang, 1988). It has been reported that the intracellular concentration of ninhydrin-positive compounds also decreases as a consequence of hyposmotic stimulus, suggesting that amino acids may contribute to the volume regulatory process (Roy & Sauvé, 1987). The specific free amino acids implicated in cell volume regulation and the features of the osmotically activated amino acid efflux in MDCK cells have not been investigated. In the present study we examined the free amino acid content in MDCK cells and their release in response to decreases in external osmolarity. The properties of the volume-sensitive release of free amino acids were examined using labeled taurine, which due to its metabolic inertness is particularly convenient for this purpose.

Materials and Methods

Solutions

Krebs-HEPES (isosmotic solution) contained (in mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.17, glucose 10, HEPES 25, adjusted to pH 7.6 with NaOH. Hyposmotic solutions were prepared by reducing the concentration of NaCl to obtain the required osmolarity. In some experiments solutions with reduced osmolarity (38.5 mM NaCl) were made isosmotic with sucrose.

Cell Cultures

The MDCK line was obtained from the American Type Culture Collection (MDCK, CCL-34) (Madin & Darby, 1958) and cloned. Cells were grown at 36°C in disposable plastic tissue culture flasks (Costar 3150, Cambridge, MA), with air 5%/CO_2 95% in humid atmosphere and Dulbecco's Modified Eagle Medium with Earle's salt (GIBCO, 430-1600, Grand Island, NY), 100 U/ml penicillin. 100 μg/ml of streptomycin and 10% calf fetal serum (GIBCO 617). Cells were harvested with trypsin-EDTA (GIBCO 540) and plated at confluence on 35-mm plastic petri dishes or in multidishes of 24 wells (Linbro Chemical, New Haven, CT). In most experiments, cells were between 60–80th passage.

Amino Acid Content

The endogenous free amino acid content of MDCK cells was measured in extracts prepared in 70% ethanol. The analysis was carried out after amino acid derivatization with O-phthaldialdehyde by reversed phase HPLC, according to Geddes and Wood (1984) and to Rajendra (1987) in a Beckman chromatographer equipped with an Ultrasphere column.

Release

For release experiments using ^{3}H-taurine, cells were preloaded with the labeled amino acid (1 μCi/ml) in the culture medium. After 30 min of incubation, the medium was replaced by Krebs solution. For studies on the time course of ^{3}H-taurine release, cells grown in petri dishes were superfused according to the procedure of Drejer, Honore and Schousboe (1987). Cells were superfused at a rate of 1 ml/min. After a wash period of 8 min at which time ^{3}H-taurine efflux baseline was attained, samples were collected every min. At the time indicated at each experiment, the superfusion medium was replaced by analogous medium with reduced osmolarity. At the end of the superfusion, radioactivity in samples and that remaining in cells was measured by scintillation spectrometry. Results are expressed as fractional rate constants calculated according to the equation

$$f = \frac{A}{t + At}$$

where A represents the radioactivity lost in the interval t and At represents the difference between total radioactivity accumulated by cells during loading and radioactivity released at a given perfusion time (Hopkin & Neal, 1971).

For experiments to measure the release of endogenous free amino acids and all other experiments with ^{3}H-taurine, cells grown in 24-well plates were used. Cell loading was carried out as described above and after 30 min of incubation with labeled taurine, the culture medium was replaced by Krebs-HEPES solution. Cells were washed three times with Krebs-HEPES isosmotic solution and incubated for 15 min (three periods of 5 min each). Media from this incubation period were pooled and corresponded to basal stimulation. Cells were then incubated with medium of reduced osmolarity for a period of 15 min. Results are expressed as a percentage of total radioactivity accumulated during loading, excluding the washing period, i.e., radioactivity in basal and stimulated release plus radioactivity remaining in the cells at the end of the experiment.

To examine the effect of drugs, the tested compounds were added at the end of the loading period and were present throughout the washing and incubation periods. When solutions of drugs were prepared using solvents different from water, controls were exposed to the same concentration of the solvent used.

Results

Free Amino Acid Content

The free amino acid content of MDCK cells cultured in an Eagle's medium is shown in Table 1. Glycine was the most abundant, followed by glutamic acid and alanine. Taurine concentration in cells grown in the culture medium without taurine was 41.5 nmol/mg protein, but when cells were cultured in a medium containing taurine, the endogenous levels of taurine markedly increased. Addition of taurine to the culture medium at a concentration of 150 μM, which is within the range of plasma levels, resulted in a threefold increase of intracellular taurine. When the concentration of external taurine was 5 mM, an increase of about sixfold was observed after 24 hr (Table 1). Coincident with the increase in taurine content, a decrease in the major free amino acids occurred. Reductions in glycine, alanine glutamic acid and β-alanine levels were observed, whereas the concentration of other amino acids did not change (Table 1).

Table 1. Free amino acid content of MDCK cells grown in the presence of 5 mM taurine

Amino acid	Control	
	nmol/mg protein	Taurine, 5 mM
Gly	84.9 ± 4.8	61.5 ± 3.62
Glu	79.4 ± 3.0	58.3 ± 3.38
Ala	72.8 ± 3.29	56.0 ± 7.50
Tau	41.2 ± 0.92	254.6 ± 16.3
Gln	25.2 ± 0.42	24.8 ± 0.32
Ser	21.4 ± 1.60	
Lys	19.8 ± 0.94	16.9 ± 1.06
Thr	16.8 ± 1.00	
β-Ala	12.4 ± 0.35	9.53 ± 0.32
Arg	12.0 ± 0.60	
His	10.8 ± 0.64	
Val	8.4 ± 0.54	8.7 ± 0.46
Leu	7.3 ± 0.42	
Phe	6.2 ± 0.34	6.1 ± 0.66
Ile	5.4 ± 0.55	5.6 ± 0.26
Tryp	2.1 ± 0.098	2.0 ± 0.11

Cells were cultured in Eagle's medium for two days. At this time 5 mM taurine was added to the medium. One day later, the free amino acid content of cells grown in the presence or absence of taurine was determined by HPLC. Results are means ± SEM of eight experiments.

The initial endogenous concentration of free amino acids was 426 nmol/mg protein (Table 1), and the protein content of 360 pg/cell was found in our cultures. Assuming an average volume of 2.2 pl/cell (McRoberts, Tran & Saier, 1983; Mills, 1987; Roy & Sauvé, 1987), a figure of 6 μl/mg protein was calculated as cell volume. The concentration of the free amino acid pool is therefore 69.8 mM.

Volume-Sensitive Release of Taurine and Amino Acids

Cells superfused with isosmotic medium (310 mOsmol) released previously accumulated ^{3}H-tau-

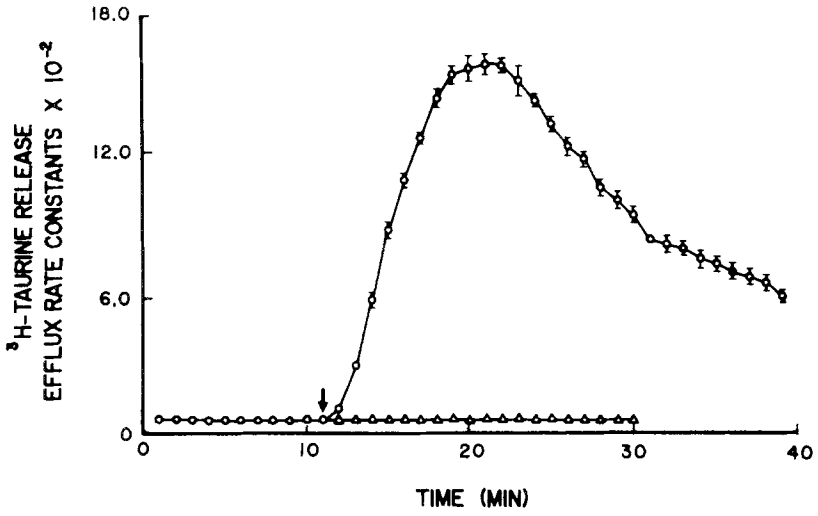

Fig. 1. Time course of ^{3}H-taurine release from MDCK cells evoked by reduced osmolarity. Cells were preloaded, washed and superfused as described in Materials and Methods. At the time indicated by the arrow, the superfusion medium (isosmotic Krebs-HEPES) was replaced by a medium with reduced osmolarity (150 mosmol, 38 mM NaCl) (○), or with a medium with the same NaCl concentration but made isosmotic with sucrose (△). Results are expressed as efflux rate constants as defined in Materials and Methods and correspond to means ± SEM of four experiments

rine at an efflux rate of 0.6% per min. Superfusion with a medium of reduced osmolarity (50%) evoked an immediate and dramatic increase in the rate of taurine efflux (Fig. 1). The peak release was attained after 10 min of superfusion, and then ^{3}H-taurine efflux declined despite the persistence of the hyposmotic stimulus (Fig. 1). The release of ^{3}H-taurine from MDCK cells was clearly associated with reductions in osmolarity. A release of 9.0% of accumulated ^{3}H-taurine was observed during incubation in isosmotic medium (310 mOsmol) for 15 min. Decreasing osmolarity from 310 to 263 mOsmol, which represents a reduction of only 15%, induced an increase in the efflux of ^{3}H-taurine to 24% in the same period. Lowering osmolarity by 25%, to 232 mOsmol, enhanced taurine efflux to 52.5%, and in solutions of 150 mOsmol (50% hyposmotic) the release of taurine increased to 75.9% (Fig. 2).

Reductions in osmolarity were obtained by decreasing the concentration of NaCl in the solutions, but the release of taurine in hyposmotic conditions was not due to the reduction in NaCl, since no increase in taurine efflux occurred in solutions with low NaCl but made isosmotic with sucrose (Fig. 1).

Endogenous free amino acids and taurine were also released as a result of decreased osmolarity. All amino acids responded to hyposmolarity, but the magnitude of the release differed considerably. Glutamic acid, β-alanine and taurine showed the largest response. Reducing osmolarity to 0.85, 0.75 or 0.5, resulted in the release of 20–30%, 40–50% and 75–80%, respectively, of the endogenous concentration (Fig. 3*A*). In the same conditions of reduced osmolarity, the release of alanine, glycine and serine corresponded to 18–22%, 30–40% and 57–60% of the original content in cells (Fig. 3*B*). A third group of amino acids, which includes isoleucine, phenylalanine and valine, was much less sensitive to hyposmolarity (Fig. 3*C*).

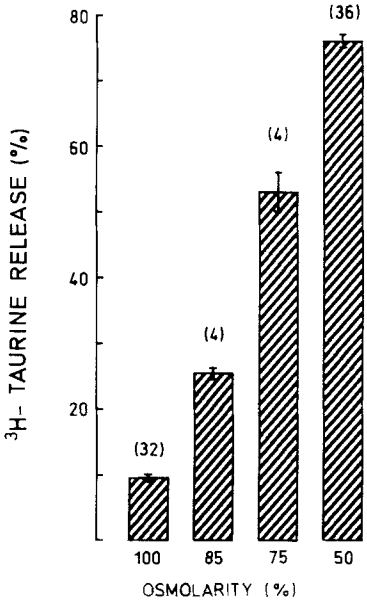

Fig. 2. Effect of decreasing osmolarity on the release of ^{3}H-taurine from MDCK cells. Cells grown in 24-well plates were preloaded, washed and incubated in isosmotic Krebs-HEPES (100% osmolarity) for 15 min. Then this medium was replaced by a medium of reduced osmolarity: 85, 75 and 50% of the original 100% isosmotic solution. Results represent ^{3}H-taurine released in 15 min of exposure to the hyposmolar conditions, expressed as a percentage of total radioactivity accumulated by cells as described in Materials and Methods. In this and the following figures, results are means ± SEM of the number of experiments indicated

Effect of External Ions on the Release of ^{3}H-Taurine

Removal of external calcium and addition of cobalt to the incubation solutions did not affect the release of taurine either in isosmotic or in hyposmotic conditions. Also, calcium-depleted cells grown in a calci-

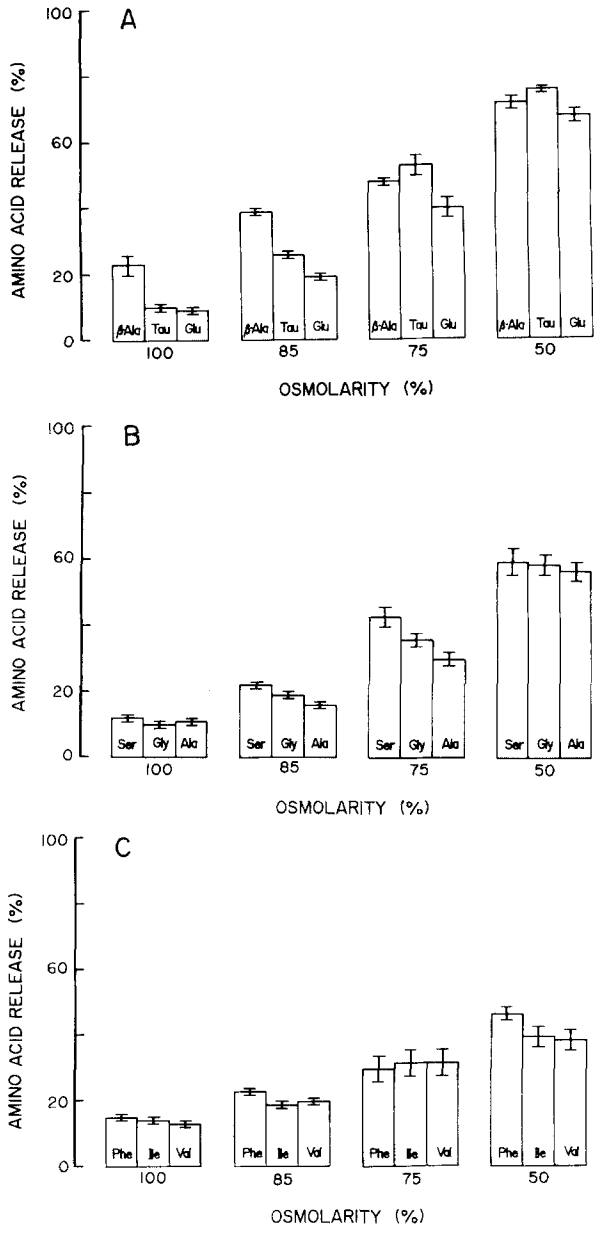

Fig. 3. Effect of decreased osmolarity on free amino acid release from MDCK cells. Cells were incubated in isosmotic medium (100% osmolarity) or in media of decreased osmolarity as indicated for 15 min. The concentration of free amino acid released during incubation was determined by HPLC as described in Materials and Methods. Results are expressed as a percentage of the amino acid concentration present in nonincubated cells. (*A*) Release of β-alanine, taurine and glutamic acid. (*B*) Release of serine, glycine and alanine. (*C*) Release of phenylalanine, isoleucine and valine. Results are means ± SEM of four experiments

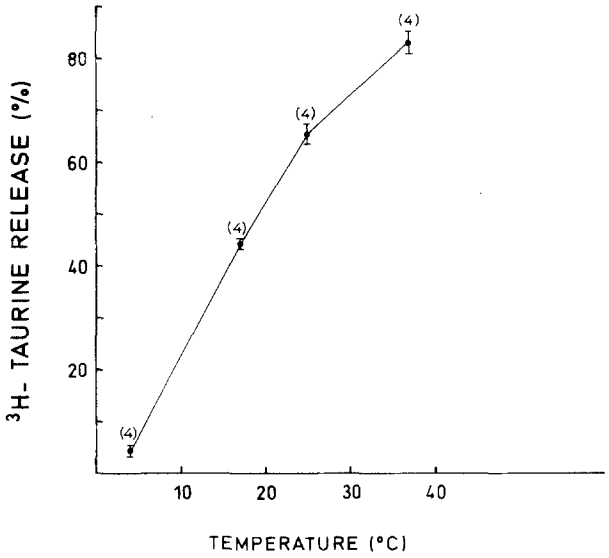

Fig. 4. Effect of temperature on ^{3}H-taurine release evoked by hyposmolarity. Cells were loaded, washed and incubated as described in Fig. 2 in hyposmolar medium (50%) at the temperatures indicated. Results are expressed as a percentage of total radioactivity accumulated by cells

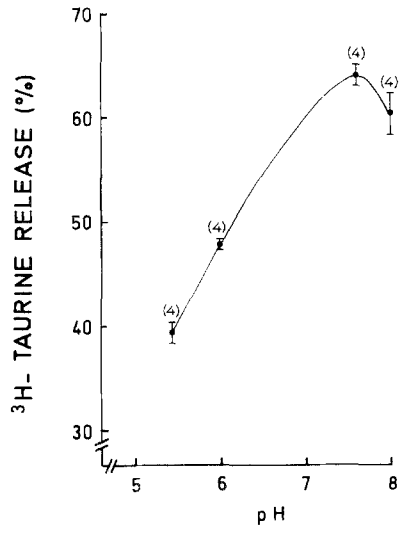

Fig. 5. Effect of pH on ^{3}H-taurine release evoked by hyposmolarity. Cells were loaded, washed and incubated as described in Fig. 2 in hyposmolar medium (50%) at the pH indicated

um-free culture medium showed responses identical to those of nondepleted cells. Replacement of sodium by choline and of chloride by gluconate had no effect on the volume-sensitive release of taurine (*results not shown*).

Effect of Temperature and External pH

The release of taurine in response to hyposmotic conditions was clearly temperature dependent (Fig. 4). At 25°C the release was 78.6% of that observed at 37°C. At 15 and 4°C taurine release was only 52 and 5%, respectively, from the release occurring at 37°C (Fig. 4). Taurine efflux evoked by a decrease in osmolarity was reduced at low pH values and increased at high pH values (Fig. 5).

Effect of Agents Disrupting the Cytoskeleton

The effect of colchicine (0.5 mM) and cytochalasin B (20 μM) on the volume-sensitive release of taurine was examined by preincubating cells in the presence of the drugs for 30 min, followed by exposure to the disrupting agents throughout the experimental procedure. This treatment had no effect on the release of taurine evoked by decreased osmolarity, which was 78.4 ± 1.41% (n = 12) in controls, 81.7 ± 1.18% (n = 8) in the presence of colchicine and 75.4 ± 1.76% (n = 12) in the presence of cytochalasin B.

Effect of Inhibitors of Ionic Fluxes Associated with Regulatory Volume Decrease

The effects of compounds or conditions known to inhibit ionic fluxes associated with volume adjustment were examined on the volume-sensitive release of taurine in order to investigate a possible link between these ionic fluxes and the swelling-evoked efflux of taurine. The inhibitors of the $Na^+/K^+/2Cl^-$ cotransporter furosemide and bumetanide did not influence the volume-sensitive release of taurine (*data not shown*). The stilbene derivative inhibitor of Cl^- channels, DIDS, at a concentration of 100 μM, markedly reduced the release of taurine evoked by 50% hyposmolar solutions. A less marked effect of DIDS was observed in 75% hyposmolar solutions (Figs. 6 and 8). Other inhibitors of chloride channels, or of stretch-activated channels, gadolinium, DPC and apamine did not influence the volume-sensitive release of taurine (Fig. 6).

Barium, tetraethylammonium (TEA) and quinidine, blockers of potassium channels, are known to inhibit the regulatory volume decrease in some cells (Grinstein et al., 1983; Hoffmann, Simonsen & Lambert, 1984; Hazama & Okada, 1988) and K^+ conductances in MDCK cells (Bolivar & Cereijido, 1987). None of these compounds affected the release of ^{3}H-taurine in isosmotic conditions, but the release induced by decreasing osmolarity to 150 mOsmol was inhibited by TEA (16%) and particularly by quinidine (67%) (Fig. 7). Contrary to that observed for DIDS, the inhibitory effect of quinidine was higher in 75% hyposmotic solutions, in which quinidine practically abolished the volume-sensitive release of taurine (Fig. 8). The inhibitory actions of DIDS and quinidine were additive (Fig. 8). These drugs also inhibited the release of endogenous amino acids evoked by hyposmolarity (50%) (Table 2).

Since quinidine but not other K^+-channel blockers inhibited taurine release, experiments were devised to investigate the possible mechanism of this inhibition. A decrease in intracellular K^+ subsequent to swelling-activated K^+ channels may be the signal for taurine release, and therefore, the inhibition of K^+ fluxes by quinidine with subsequent maintenance of high K^+ levels may reduce taurine efflux. To investigate this possibility the K^+ gradient was dissipated with valinomycin (1 μM), thus creating an alternate K^+ efflux pathway insensitive to quinidine.

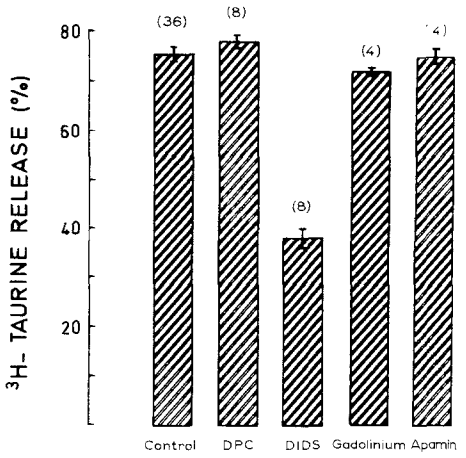

Fig. 6. Effect of inhibitors of Cl^- and stretch-activated channels on the release of ^{3}H-taurine evoked by decreased osmolarity. Cells were loaded and incubated as described in Materials and Methods. Drugs used were DIDS (100 μM), diphenylamino-2-carboxylate (100 μM), gadolinium (10 μM) and apamin (0.1 μM). Cells were treated with drugs at the end of the loading period for 15 min and were present in isosmotic and hyposmotic media. Bars represent the release of ^{3}H-taurine (%) evoked by exposure to hyposmotic medium (50%)

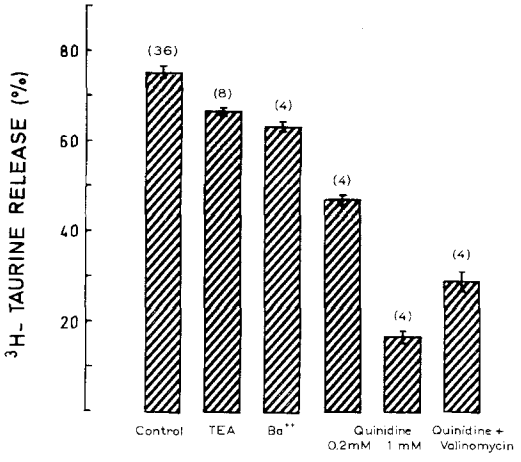

Fig. 7. Effect of K^+-channel blockers on the release of ^{3}H-taurine evoked by decreased osmolarity. The experimental procedure was as described in Fig. 6. Drugs used were TEA (10 mM), barium (15 mM) and quinidine (0.2 and 1 mM), and quinidine (1 mM) plus valinomycin (1 μM). Bars represent the release of ^{3}H-taurine (%) evoked by hyposmolar solutions (50%)

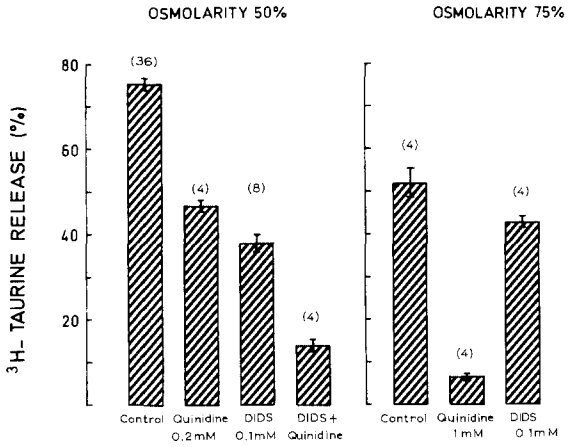

Fig. 8. Effect of DIDS and quinidine on the release of ^{3}H-taurine evoked by hyposmolar 50 and 75% media. Loading, washing and incubation with drugs were as described in Fig. 6. Concentration of drugs was as indicated. When DIDS and quinidine were used together, their concentration was 0.1 and 0.2 mM, respectively

Table 2. Effect of DIDS and quinidine on the release of free amino acids from MDCK cells evoked by hyposmolarity

Amino acid	Release (%)		
	Control	DIDS	Quinidine
Taurine	76.0 ± 1.3	38.0 ± 2.0	17.3 ± 1.3
β-Alanine	72.2 ± 2.0	48.2 ± 1.3	46.1 ± 1.2
Glutamic acid	68.3 ± 1.5	39.3 ± 1.7	39.4 ± 0.9
Serine	60.3 ± 4.2	25.3 ± 2.5	27.4 ± 0.8
Alanine	57.4 ± 2.8	23.2 ± 1.0	21.8 ± 1.0
Glycine	58.6 ± 2.9	31.5 ± 1.4	20.2 ± 0.9
Phenylalanine	47.3 ± 1.8	15.4 ± 1.2	30.0 ± 3.4
Isoleucine	39.6 ± 3.4	13.2 ± 2.0	13.6 ± 1.5
Valine	39.0 ± 2.9	17.1 ± 0.4	

Cells were incubated with DIDS (100 μM) or quinidine (1 mM) for 20 min and then exposed to media of reduced osmolarity (50%) containing the same concentration of the drugs. Amino acid release is expressed as a percentage of the endogenous content of cells. Results are means ± SEM of four experiments.

Under these conditions quinidine still inhibited taurine release by 53% (Fig. 7).

It has been reported that quinidine decreases amino acid transport in hepatocytes (Wondergem & Castillo, 1988), and therefore, the inhibitory action of quinidine on taurine release may be exerted on a transport mechanism associated with taurine release. Taurine is accumulated by a Na^+-dependent process. Reduction in external Na^+ concentration from 118 to 6 mM resulted in an inhibition of ^{3}H-taurine uptake of more than 70% (Fig. 9). Quinidine (1 mM) reduced ^{3}H-taurine uptake by MDCK cells by about 60%, whereas DIDS and TEA had no effect on this process (Table 3).

Discussion

The results of the present study confirm the observation of Roy and Sauvé (1987) on the loss of ninhydrin-positive compounds from MDCK cells exposed to hyposmotic solutions. All the main constituents of the free amino acid pool, i.e., glycine, alanine, taurine, serine and glutamic acid, appear to be involved in the volume regulatory process. Taurine concentration is relatively low in cells grown in current culture media that do not contain taurine. This condition, however, may not reflect the physiological situation, since plasma and extracellular fluids always contain taurine, which is avidly accumulated by cells. Cell taurine pool results from both biosynthesis and active uptake, which contribute in proportions varying in the different tissues (Huxtable & Lippincott, 1982). The present results show that MDCK cells are able to synthesize taurine to some extent, but the taurine pool is substantially increased by active accumulation. A decrease in the concentration of other free amino acids is observed as taurine accumulates, suggesting the existence of an exchangeable pool of the main free amino acids in MDCK cells. This pool is probably the one involved in cell volume regulation.

The intracellular content of free amino acids decreases in response to hyposmotic stimulation. Upon exposure to a 50% hyposmotic solution the initial endogenous content of about 70 mM decreases to less than 25 mM. Therefore, the release of free amino acids contributes with about 40 mOsmol to the volume adjustment of MDCK cells facing an osmolarity reduction of 150 mOsmol.

The release of labeled taurine, taken as representative of free amino acid release evoked by hyposmolarity, is rapid and proportional to reductions in osmolarity. The time course of taurine release closely follows the volume change in the cells, both in onset and offset. The declining phase of taurine efflux is not due to depletion of the intracellular pool since at the end of the volume regulatory process cells still contain about 20% of taurine. Also, taurine efflux in the decreasing phase for the interval of 20–30 min has a constant slope despite the large differences in intracellular taurine between the first and last points of the curve at this interval. This is an indication of a signal terminating the release process. Roy and Sauvé (1987) reported that no loss of ninhydrin-positive compounds is observed when osmolarity is reduced by 20%. Using labeled taurine or HPLC for determination of free amino acid levels,

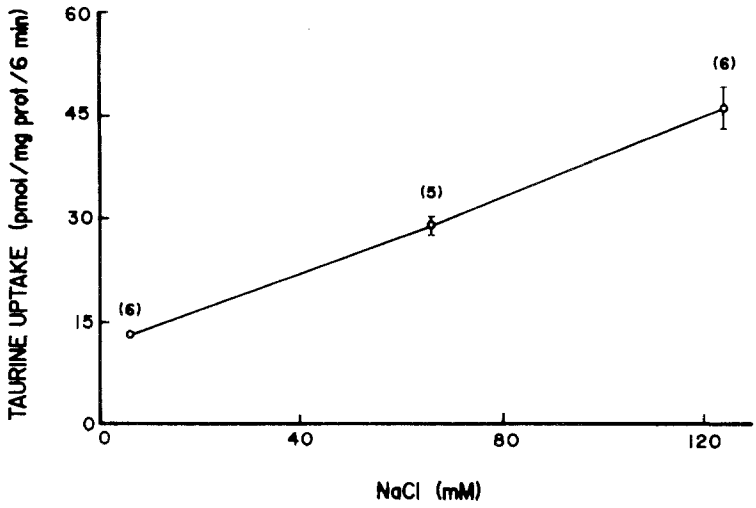

Fig. 9. Effect of Na^+ on ^3H-taurine uptake by MDCK cells. The concentration of Na^+ in the medium was decreased, replacing NaCl by choline chloride. Incubation conditions were as described in Table 3. Results are means ± SEM of four experiments

Table 3. Effect of inhibitors of the volume-sensitive release of taurine on ^3H-taurine uptake by MDCK cells

	cpm × 10^3/mg protein
Control	75.1 ± 0.07
Quinidine 1 mM	30.6 ± 0.29
TEA 10 mM	76.4 ± 1.92
DIDS 0.1 mM	81.2 ± 0.80

Cells were incubated for 30 min with ^3H-taurine (1 μCi/ml) in the presence or absence of the drugs. After incubation cells were washed and accumulated radioactivity was measured by scintillation spectrometry. Results are means ± sem of four experiments.

a volume-sensitive release could be observed in media containing 263 mOsmol with a reduction of only 15% in the osmolarity, although the magnitude of the response of the various amino acids was different and this difference persisted at all the osmolarities examined. The reason for this difference is still unclear. The largest release observed for some amino acids may be due to the accessibility of endogenous pools to the volume-sensitive efflux process, or alternatively, release of amino acids may occur through more than one mechanism with a different threshold to activation by volume changes.

Free amino acids, together with K^+ and Cl^-, are the main osmolytes mobilized during volume regulation in most cells (Gilles, 1988; Macknight, 1988), and this also seems to be the case for MDCK cells (Roy & Sauvé, 1987). Ion transport systems proposed to be directly involved in volume regulation are either electroneutral ion cotransporters or conductive K^+ and Cl^- channels. Although this question has not been clarified in MDCK cells, the failure of furosemide to modify K^+ and Cl^- changes in cells after hyposmotic challenge (Roy & Sauvé, seems to exclude the involvement of K^+/Cl^- or $Na^+/K^+/2Cl^-$ electroneutral carriers which are sensitive to this drug, suggesting the involvement of channel-mediated fluxes.

The possibility of a connection between ionic and organic osmolytes has been addressed in the present study by investigating effects of blockers of volume-associated ionic fluxes on free amino acid release. Antagonists of K^+ channels excluding quinidine had only marginal effects on taurine efflux. In MDCK cells, quinidine inhibits the swelling-activated K^+ efflux, whereas other blockers of K^+ channels were ineffective (Roy & Sauvé, 1987). Similarly, quinidine but not barium, inhibits the volume-sensitive fluxes of sorbitol in cultured renal cells (Siebens & Spring, 1989). All these observations suggest the involvement of a quinidine-sensitive mechanism in the release of osmolytes during the volume regulatory process. This may be the activation of a channel which, however, would be insensitive to blockers of other well-characterized K^+ channels. A number of stretch-activated channels has been recently described, although none in MDCK cells, but they have not been pharmacologically characterized. It cannot be excluded, therefore, that one of those channels sensitive to quinidine is directly or indirectly involved in the release of taurine. The observation that stimulating K^+ efflux with valinomycin does not counteract the inhibitory effect of quinidine on taurine release supports the notion that a coupled K^+-taurine release occurs through a quinidine-sensitive pathway. In this respect it should be noted that besides the inhibition of K^+ channels, quinidine has numerous other effects, which include decreases in membrane fluiditiy (Needham, Dodd & Houslay, 1987), blockade of Na^+ and Ca^{2+} channels (Honerjager et al., 1986) and inhibition of Na^+/H^+ or Na^+/Ca^{2+} exchangers (Parker, 1981).

Since quinidine inhibited the Na^+-dependent taurine uptake, its effect on taurine release may be alternatively explained through an action on the Na^+-dependent carrier working outwards. However, the failure of Na^+ omission to affect taurine release observed in the present study would argue against the involvement of such a carrier on the osmolarity-sensitive release of taurine. Also the absence of homoexchange, i.e., the stimulation of labeled taurine release by external unlabeled taurine (*results not shown*), does not support the idea of a mechanism for release involving the transport carrier. Activation of taurine leak pathays by hyposmolarity has been suggested in Ehrlich ascites tumor cells (Hoffmann & Lambert, 1983) and in fish erythrocytes (Fincham, Wolowyk & Young, 1987).

The inhibitory effect of DIDS on the osmolarity-sensitive release of taurine suggests an association of taurine efflux also with DIDS/SITS-inhibitable conductances. An ionic transport system with relatively low equilibrium potential activated upon exposure to hyposmotic conditions in MDCK cells (Völkl et al., 1988), may correspond to a SITS-inhibitable unselective ion channel of the type that has been described in cultured opossum kidney cells (Ubl, Murer & Kolb, 1988) or in mouse B lymphocytes (Bosma, 1989). The lack of effect of other inhibitors of chloride channels or of stretch-activated channels in different cells (Yang & Sachs, 1989) may be interpreted either as considering that the effect of DIDS on taurine release does not reflect its connection with chloride channels or stretch-activated pores or, alternatively, that these channels, if present in MDCK cells, are only sensitive to DIDS. Along this line, a recent report describes a volume-evoked Cl^- efflux sensitive to DIDS but not to DPC in MDCK cells (Rothstein & Mack, 1989). This restricted sensitivity to distilbene compounds has also been observed in the multiple conductance pore of mice lymphocytes (Bosma, 1989). A definite answer to this question must await the identification and pharmacological characterization of ionic conductances associated with volume regulatory processes in MDCK cells. The effects of DIDS and quinidine were additive, suggesting that two different mechanisms may be involved in the efflux of taurine induced by swelling. The different sensitivity of the two drugs to decreases in osmolarity suggest that these mechanisms may be activated at a different stimulus intensity.

Besides inorganic ions, organic osmolytes are also involved in volume regulation in renal cells. Volume-sensitive sorbitol fluxes have been characterized, exhibiting many similarities with the hyposmolarity-stimulated taurine efflux (Bagnasco et al., 1988; Nakanishi, Balaban & Burg, 1988). The results of the present study strongly support a contribution of free amino acids to this process in MDCK cells. Free amino acids are involved in mechanisms of volume control in cells and tissues of vertebrates and invertebrates naturally facing changes in osmolarity, but it is only recently that their implication in such a role in mammalian tissues has been considered. The structure, function and antigenicity of MDCK cells are remarkably similar to those of cells from the distal nephron (Cereijido et al., 1988), a segment intimately involved in osmotic regulation. Furthermore, plasma membrane of MDCK cells contains a large (220-pS) potassium channel (Bolivar & Cereijido, 1987) of the type that participate in the response to cell swelling (Sachs, 1990). Therefore, MDCK cells represent a very convenient model for further exploring the role of free amino acids in cell volume regulation.

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