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Volume-Activated Taurine Permeability in Cells of the Human Erythroleukemic Cell Line K562

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The effects of hypotonic shock on cell volume, taurine influx and efflux were examined in the human erythroleukemic cell line K562. Cells exposed to hypotonic solutions exhibited a regulatory volume decrease (RVD) following rapid increases in cell volume. Cell swelling was associated with a increased taurine influx and efflux. The volume-activated taurine pathway was Na^+-independent, and increased in parallel with increasing cell volume. The chloride channel blocker, 2,5-dichlorodiphenylamine-2-carboxylic acid (DCDPC), completely blocked the volume-activated taurine influx and efflux, while [dihydroindenyl)oxy]alkanoic acids (DIOA) and 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), an anion exchanger and anion channel blocker, respectively, also inhibited significantly. These results suggest that taurine transport is increased in response to hypotonic stress, which may be mediated via a volume-activated, DCDPC-sensitive anion channel. © 1996 Wiley-Liss, Inc.

Most cell types have mechanisms to regulate their volume in response to osmotic perturbation. Taurine (β-aminoethanesulphonic acid) is an organic osmolyte which is present in high concentrations in many types of vertebrate and invertebrate cells (Fincham et al., 1987; Kirk and Kirk, 1993; Hoffman and Lambert, 1983; Kimelberg et al., 1990). Regulatory volume decrease (RVD) following hypotonic stress has been demonstrated to occur in parallel with taurine efflux (Fincham et al., 1987). Under isoosmotic conditions in many cell types, taurine is taken up by a Na^+-dependent system unique for β-amino acids (Fincham et al., 1987). However, during RVD, the Na^+-dependent amino acid influx pathway is inhibited, while the Na^+-independent amino acid transport is increased (Fincham et al., 1987).

Volume-activated taurine influx has been suggested to share a common transport pathway with other anions or organic osmolytes (Kirk et al., 1992), but at least three transport pathways have been implicated in taurine influx: (1) The band 3 anion exchanger system, based on inhibition by DIDS, which also inhibits chloride channels (Musch et al., 1994); (2) an Na^+-independent amino acid channel (Ballatori et al., 1994); and (3) a volume-activated chloride channel (Kirk et al., 1992; Lambert and Hoffmann, 1993).

Although volume-regulatory processes have been studied in red blood cells of several species, little is known regarding volume-activated taurine transport in mammalian red blood cells. A small volume-activated taurine flux has been demonstrated in horse red cells (Gibson et al., 1993), but normal human red cells do not exhibit volume-activated taurine fluxes (Culliford et al., 1995). Previous studies have demonstrated that K562 cells represent an early differentiation stage of the granulocyte lineage (Klein et al., 1976). In addition, K562 cells can be induced by various mutagens to differentiate toward normal erythroid cells that express globin genes (Cioe et al., 1981), amino acid transport systems (Vadgama et al., 1991), and Ca^{2+}-activated K^+ channels (Huang and Ellory, 1994). The present study investigated volume-activated taurine transport in the human erythroleukemic cell line K562 as these cells are a useful model to study membrane transport properties of human erythrocytes during distinct stages of differentiation. Our results suggest that taurine influx and efflux are increased by hypotonic shock, via anion permeability pathways.

MATERIALS AND METHODS

Cell culture

The human erythroleukemic cell line K562 (a gift from Dr. M. Pondel, Department of Pathology, Oxford, U.K.) was maintained in RPMI 1640 medium (GIBCO, Renfrewshire, Scotland, U.K.) containing 5% foetal calf serum, 1 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, at 37°C in a 5% CO_2 incubator.

Taurine efflux measurement in K562 cells

K562 cells in suspension were harvested and washed three times with supplemented RPMI medium, and preloaded with ^{3}H-taurine (0.037 MBq/ml) for 15 min. Cells were subsequently washed four times with ice-cold RPMI medium, then transferred to one of the following conditions: (1) Isoosmotic RPMI, (2) 50% RPMI,

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or (3) 50% RPMI with inhibitors. Taurine efflux was measured over a 10 min period. Time 0 is defined as the starting point when cells were added into the above solutions. Aliquots of the cell suspensions were removed at time intervals (1, 2, 5, 10 min) and immediately centrifuged at room temperature for 5 sec at 10,000g. Aliquots of the supernatant were removed for scintillation counting. All results were calculated as the rate constant (k, h^{-1}) for taurine efflux [ln(C∞-Cs/C∞-Co)/t], where C∞, Cs, and Co are the extracellular radioactivities at *time infinity, time t,* and *time zero,* respectively. Rate constant k is given by the slope of the line [ln(C∞-Cs/C∞-Co)/t].

Taurine influx measurement

The method used for influx studies was previously described by Kirk et al. (1992). Briefly, 8–12 microcentrifuge tubes were prepared, containing 0.1 ml RPMI medium, and 1.25 mM furosemide (to block all influx), which was layered over 0.4 ml of dibutylphthalate/silicone oil mixture. K562 cells were added to either isoosmotic or 50% diluted RPMI containing ^{3}H-taurine (0.037 MBq/ml) and 1 mM cold taurine. At the appropriate time intervals, an aliquot of the cell suspension (0.1 ml) was dispensed into each micro-centrifuge tube. Following addition of cells to the tube, the sample was centrifuged to allow the cells to sediment below the oil, and thereby terminate the flux (Kirk et al., 1992). Timed samples were subsequently rinsed with water, and the oil layer aspirated. The cell pellets were lysed with Triton X-100, deproteinized by the addition of 5% (w/v) TCA and centrifuged. Radioactivity was measured by using β-scintillation spectrometry.

Cell volume measurement

Cell volume was measured with a Coulter Counter Multisizer (Coulter Electronics, Luton, Beds, U.K.) interfaced with a multichannel analyzer. Median cell volume of cell suspensions was determined according to the method of Segel et al. (1981) from the Channelyzer frequency distribution with a confined major K562 cell peak. K562 cells were diluted into isoosmotic medium and aliquots immediately taken for cell volume measurement. Subsequently the medium was diluted 50% and further aliquots taken at 2–3 min intervals for successive volume measurements. Flux measurements were made separately with cells from the same culture. Cell volume is expressed as relative volume (measured volume/isotonic volume for each experiment).

Chemicals and solutions

^{3}H-taurine was obtained from Du Pont-New England Nuclear (Stevenage, Herts, U.K.). Furosemide, 4.4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), 4-acetamino-4′-isothiocyanatostilbene-2-2′-disulfonic acid (SITS), and 4-4′dinitro-2-2′-stilbenedisulfonate (DNDS) were purchased from Sigma Ltd. (Dorset, U.K.). [dihydroindenyl)oxy]alkanoic acid (DIOA) was from Semat Technical (St. Albans, Herts, U.K.). 2,5-dichlorodiphenylamine-2-carboxylic acid (DCDPC) was a gift from Dr. H. Englert, Hoechst, Frankfurt, Germany, and 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) was a gift from Dr. K. Kirk. The normal saline used during Na^+-dependence experiments contained 134.4 mM NaCl, 5.6 mM KCl, 15 mM MOPS, 10 mM glucose, 0.24 mM $MgCl_2$ (pH 7.4). All solutions were sterilized before use.

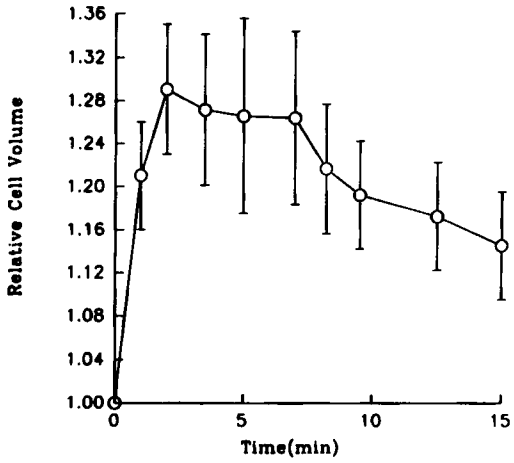

Fig. 1. Cell volume changes in K562 cells exposed to hypotonic conditions. K562 cells suspended in RPMI medium were exposed to 50% hypotonic shock. Cell volume was measured as described in Materials and Methods.

Protein measurement

The amount of total cell protein was determined by the method of Bradford (1976) with bovine serum albumin (Bio-Rad, Hemel Hempstead, Herts, U.K.) as a standard.

Data analysis

Data are presented as means ± standard error, with a significance level of $P < 0.05$ as determined by paired t-test.

RESULTS

Cell volume changes

Reducing the osmolarity of the suspension medium from 300 mosM to 150 mosM/KgH_2O (50% hypotonic shock), increased the peak cell volume by ~30 ± 11% (n = 5) as compared to isoosmotic conditions (Fig. 1). Although the cells rapidly increased their cell volume, the maximal cell volume was obtained 3–5 min after exposure to hypotonic solutions. The rapid cell swelling was followed by regulatory volume decrease (RVD). Cell volume was reduced by 11% from peak cell volume within 9 min. After 15 min, cell volume was 15% above isoosmotic conditions. We also tried incubating cells with 300 μM DCDPC before a 50% hypotonic shock. Under this condition, cell volume was reduced only by 4 ± 2% (n = 3) from peak cell volume within 9 min.

Taurine influx

Taurine influx was measured at 37°C as a correlate of the degree of hypotonicity (0, 20, 30, or 50% hypotonic shock), over a 10 min period. The volume-activated taurine influx was dose-dependent with increasing osmotic stress (Fig. 2). Under 50% hypotonic shock, taurine influx increased five-fold compared with isoosmotic conditions.

The pathways involved in the volume-activated taurine influx were investigated with known inhibitors of

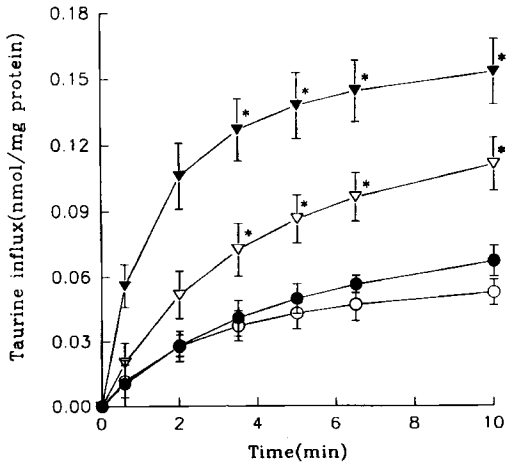

Fig. 2. Time course for ^{3}H-taurine influx in K562 cells. Cells incubated with ^{3}H-taurine were subsequently exposed to the following conditions: under isoosmotic (○), 20% hypotonic (●), 30% hypotonic (▽), or 50% hypotonic (▼), and influx of ^{3}H-taurine measured at the indicated time intervals. Data represent the means ± S.E. of results from three similar experiments. *$P < 0.05$, compared with isoosmotic conditions.

TABLE 1. Effect of anion channel blockers on volume-activated taurine influx in K562 cells[1]

Inhibitor (μm)	Volume-activated taurine influx (% of control)
DNDS (100)	113.0 ± 4.6
SITS (100)	101.6 ± 9.8
DIDS (100)	84.6 ± 12.7
DIOA (20)	62.9 ± 7.9*
NPPB (50)	58.5 ± 0.9*
DCDPC (300)	0.1 ± 5.7*

[1]At 37°C after 10 min under 50% hypotonic condition. Influx rates (mean ± S.E.) are expressed as a percentage of those measured in the absence of inhibitors. The data are averaged from 3–5 experiments.
*$P < 0.05$, compared with isoosmotic conditions.

anion transport systems. Taurine influx was suggested to be via modified band 3 in some cell types (Musch et al., 1994) as these pathways are sensitive to DIDS and SITS. In the present experiments, DIDS, SITS, as well as DNDS did not significantly inhibit volume-activated taurine influx in K562 cells (Table 1). In contrast, DIOA (20 μM), which has been reported to be an effective blocker of the anion exchanger, KCl cotransport system (Garay et al., 1988), and volume-activated taurine influx in hepatocytes (Ellory et al., 1994), significantly inhibited (37 ± 7.8%, n = 4, $P < 0.05$) the volume-activated taurine influx (Table 1). Similarly, the chloride channel blockers NPPB (50 μM) and DCDPC (300 μM) significantly ($P < 0.05$) inhibited the volume-activated taurine influx (Table 1). Complete inhibition of the volume-activated taurine influx was observed with 300 μM DCDPC.

Taurine efflux

As illustrated in Figure 3A, exposure to 50% hypotonic shock increases taurine efflux rapidly. The average taurine efflux rate constant under isoosmotic condition was 0.18 ± 0.06 h^{-1} (n = 3). Cell swelling (50% hypotonic shock) markedly increased the taurine efflux

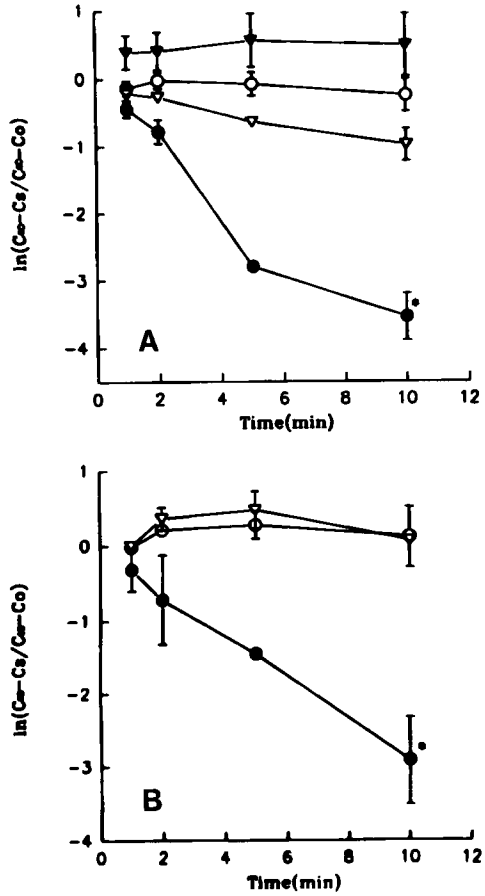

Fig. 3. Effects of DCDPC and NPPB on ^{3}H-taurine efflux. K562 cells were incubated with ^{3}H-taurine for 15 min. After loading, cells were washed three times in ice-cold RPMI medium. Cells were subsequently incubated with (**A**) isoosmotic medium (○), 50% hypotonic medium (●), 50% hypotonic medium + 33 μM DCDPC (▽), or 50% hypotonic medium + 300 μM DCDPC (▼), (**B**) isoosmotic medium (○), 50% hypotonic medium (●), 50% hypotonic medium + 50 μM NPPB (▽). Data represent the means ± S.E. of results from three similar experiments. *$P < 0.05$, with respect to isoosmotic conditions.

rate constant to 26.5 ± 8 h^{-1} (n = 3). This efflux was sensitive to DCDPC. The volume-activated taurine efflux was completely inhibited with 300 μM DCDPC, while with 33 μM, a 76 ± 2% inhibition (n = 3, $P < 0.05$) was observed. Similarly, NPPB (50 μM) significantly ($P < 0.05$) blocked the volume-activated taurine efflux (Fig. 3B).

Na^+-dependence in the volume-activated taurine influx

To examine the Na^+-dependency of the volume-activated taurine influx, the extracellular NaCl (134.4 mM) was replaced with equimolar LiCl. Subsequent exposure to hypotonic shock did not significantly alter the volume-activated taurine influx (Fig. 4). This suggests that the Na^+-dependent transport pathway is not an essential pathway for taurine influx during hypotonic stress. Similarly, taurine influx was not significantly affected by replacing Na^+ by choline or the impermeable cation N-methyl-D-glucamine$^+$ (data not shown).

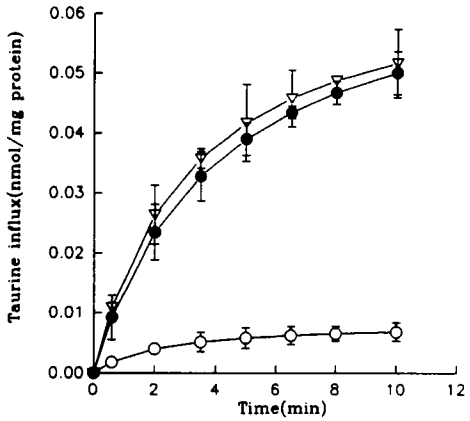

Fig. 4. ^{3}H-Taurine influx with NaCl substitution with LiCl. Cells were exposed to either isoosmotic saline (○), 50% hypotonic saline (▽), or 50% hypotonic saline with LiCl (134.3 mM) equimolar substitution from NaCl (●), and taurine influx measured as described in Materials and Methods.

DISCUSSION

The ability of cells to undergo volume regulatory responses under hypotonic conditions has been well established. K^+, Cl^-, and amino acids such as taurine, may participate in these processes. The present studies demonstrate that the human erythroleukemic cell line K562 undergoes a regulatory volume decrease (RVD) in response to hypotonic shock. Furthermore, hypotonic stress significantly increases both influx and efflux of taurine. The influx and efflux may be mediated via anion permeability pathways as both efflux and influx are sensitive to the chloride channel blockers. Replacement of Na^+ by Li^+, choline or $NMDG^+$ did not alter taurine influx under hypotonic conditions in K562 cells. This implies that taurine transport is Na^+-independent in these cells. These observations correspond to effects seen in flounder erythrocytes (Fincham et al., 1987) and Ehrlich ascites cells (Hoffmann and Lambert, 1983).

Our results showed that the time course of this volume-activated taurine influx is osmolarity-dependent, both in onset and ending. The reason for this osmolarity-dependent behavior is still unclear. In cultured MDCK kidney cells (Olea et al., 1991) and skate hepatocytes (Ballatori and Boyer, 1992), volume-activated taurine fluxes had also been showed as osmolarity-dependent. A possible explanation is that the taurine influx under hypoosmotic condition may occur through more than one mechanism with a different threshold to be activated by volume changes (Ballatori and Boyer, 1992). In K562 cells, the volume-activated taurine influx under 50% hypotonic shock gradually reach a pleateau after 5 min interval. Thus, there is a possible maximal threshold for this volume-activated taurine influx. However, the exact mechanism is still not clear.

It has been suggested that band 3 mediates volume-activated taurine transport based on the inhibition by DIDS of this volume-activated pathway (Goldstein and Brill, 1991). In the present studies, DIDS (100 μM) was not an effective blocker of the volume-activated taurine influx, although DIDS has been shown to be a potent inhibitor of anion transport band 3 in K562 cells (Dissing et al., 1984). Since DIDS did not inhibit the volume-activated taurine influx in K562 cells, it suggests that this volume-activated taurine influx in K562 cells may be not mediated via band 3. A weak inhibitory effect of DIDS on volume-activated taurine transport has also been reported in bovine chondrocytes (Hall, 1995) and mouse blastocytes (Van Winkle et al., 1994). Similarly, DNDS and SITS, which have been reported as more specific inhibitors of band 3 in various cell types (Frohlich, 1982; Cabantchik and Greger, 1992; Garay et al., 1988), did not significantly affect the volume-activated taurine influx in K562 cells. In a recent report utilizing fluxes and electrophysiological recording, volume-activated taurine transport in rat G6 glioma cells was shown to occur via a volume-sensitive anion channel, which can be blocked by NPPB and CDC (a DCDPC analogue) but not by SITS (Jackson and Strange, 1993). These results suggest that transport of taurine during hypotonic stress is unlikely to be mediated via band 3 but may be through the volume-activated anion channel.

NPPB, which is a more effective anion and chloride channel blocker than DIDS (Cabantchik and Greger, 1992), inhibits the volume-activated taurine efflux and influx in other cell types (Kirk et al., 1992; Kirk and Kirk, 1993). In K562 cells, NPPB significantly inhibited the volume-activated taurine influx and efflux. Recent reports using patch-clamp techniques showed that NPPB (at 100 μM concentration) can completely block the volume-activated chloride current in fifteen different mammalian non-excitable cell types including blood cells and lymphoma cells (Nilius et al., 1994), while NPPB (at 30 μM concentration) blocks the volume-activated chloride current in human neuroblastoma cells (Basavappa et al., 1995). Thus, the inhibitory effect of NPPB on volume-activated taurine efflux and influx in K562 cells implies that the volume-activated taurine transport may be via an anion channel. In addition, the other chloride channel blocker DCDPC (Wangemann et al., 1986; Nilius et al., 1994), was more effective as it completely blocked the volume-activated influx and efflux of taurine in K562 cells. Our results also indicated that DCDPC has the ability to largely reduce the RVD response of K562 cells under hypotonic shock. However, DIOA, a blocker of the anion channel and volume-activated taurine influx and efflux (Garay et al., 1988; Ellory et al., 1994), significantly inhibited hypoosmotically induced taurine influx in K562 cells. DIOA also was shown as a more potent taurine transport inhibitor than the chloride channel blocker dideoxyforskolin (Valverde et al., 1992). In lamprey erythrocytes, DIOA was shown to inhibit volume-activated Cl^- transport (Virkki and Nikinmaa, 1995). These results suggest that volume-activated taurine efflux and influx may be mediated via a volume-activated anion channel and there is recent electrophysiological evidence for such a channel in K562 cells (Viana et al., 1995). This specific volume-activated taurine permeable anion channel has highly binding activities to NPPB, DCDPC, and DIOA rather than the stilbene sulphonate derivatives DIDS, DNDS, and SITS. Whether this anion channel is a specific taurine channel seems unlikely since in other systems the volume-activated anion channel is also permeable to other amino acids

such as glycine (Kirk et al., 1992; Hoffmann and Simonsen, 1989).

K562 cells are used as a model system for transport studies in volume regulation of blood cells. Previously they have been shown to lack the Ca^+-activated K channel but may contain the amino acid transport systems ASC and system A (Vadgama et al., 1991; Huang and Ellory, 1994). In contrast, normal human red cells lack volume-activated taurine transport (Culliford et al., 1995). The fact that a volume-activated taurine transport system is active in K562 cells identifies another important membrane transport system which is modified or deleted during stem cell maturation.

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