Inhibition of Na^+, K^+-ATPase Activates Swelling-Induced Taurine Efflux in a Human Neuroblastoma Cell Line

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The Na^+ pump (Na^+, K^+-ATPase) has been implicated in the regulation of many cellular functions, including cell volume regulation. The effects of inhibiting Na^+ pump activity on cell volume and taurine efflux were evaluated in the human neuroblastoma cell line CHP-100. Cell volume changes monitored with the Coulter Multisizer technique and confocal microscopy showed that neuroblastoma cells exposed to ouabain swelled by 22 ± 4% (n = 5). The rapid cell swelling was followed by regulatory volume decrease (RVD). In cells treated with ouabain, ^{14}C-taurine efflux increased by 183 ± 11% compared with controls. However, cells exposed simultaneously to ouabain and hypoosmotic solution resulted in a ^{14}C-taurine efflux of 207 ± 18%. Western blot and immunofluorescence microscopy with specific monoclonal antibodies for the catalytic α isoforms of Na^+, K^+-ATPase demonstrated high levels of the ubiquitously expressed α1 and the neuronal-specific α3. Ouabain-binding data showed that CHP-100 cells express $\sim 3 \times 10^5$ pump units/cell. The present data indicate that efflux of taurine may be involved during volume recovery subsequent to blockade of Na^+, K^+-ATPase in CHP-100 cells. **J. Cell. Physiol. 174:145–153, 1998.** © 1998 Wiley-Liss, Inc.

Na^+, K^+-ATPase plays a vital role in maintaining cell volume by actively extruding Na^+ to counterbalance the colloidal osmotic pressure contributed by charged intracellular macromolecules. Maintenance of cell volume is particularly crucial in the brain, because the extracellular environment of neurons is constantly changing with secretion of neurotransmitters and/or hormones in the natural confinement imposed by a rigid cranium. Furthermore, in nerve cells, the Na^+ pump maintains high levels of intracellular K^+ and low levels of intracellular Na^+. This delicate balance between K^+ and Na^+ is a requisite for proper neuronal electrical activity (Hodgkin and Huxley, 1952; Thomas, 1972). Thus, blockade of the Na^+ pump would be expected to result in membrane depolarization, followed by cell swelling (Quinn and Pierce, 1992). This is particularly evident in pathological conditions, such as cerebral ischemia, where depleted ATP stores compromise Na^+, K^+-ATPase activity, leading to subsequent cytotoxic edema or cell swelling (Klatzo, 1994).

Neuronal cells, like most other cell types, initially swell and subsequently undergo regulatory volume decrease (RVD) in response to hypoosmotic stress. Although RVD may involve a variety of pathways, a commonly utilized pathway involves activation of separate K^+ and Cl^- channels, which leads to loss of K^+, Cl^-, and/or amino acids and the subsequent loss of water (Hoffmann and Simonsen, 1989; Sarkadi and Parker, 1991; Basavappa and Ellory 1996; Strange et al. 1996). However, recent evidence suggests that the swelling-activated anion conductance is permeable to other anions and amino acids and, in particular, to the β-aminoethanesulphonic amino acid, taurine (Banderali and Roy, 1992; Kirk et al., 1992; Jackson and Strange, 1995; Basavappa et al., 1996).

An intriguing aspect of RVD is the mode of cell swelling. Does the cell activate a similar pattern of swelling-induced taurine efflux (as described above) in response to cell swelling produced in the absence of hypoosmotic stress? Thus, in the present study, we investigated the hypothesis that 1) inhibition of the Na^+ pump leads to cell swelling, and 2) the subsequent regulation of cell volume involves activation of swelling-induced taurine efflux. The present data in the human neuroblastoma cell line CHP-100 indicate that the catalytic α1 and the neuronal-specific α3 isoforms of Na^+, K^+-ATPase are expressed and that inhibition of Na^+ pump activity

Contract grant sponsor: Wellcome Trust; Contract grant sponsor: Arthritis and Rheumatism Council.

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Received 28 October 1996; Accepted 10 June 1997

leads to cell swelling followed by increased taurine efflux.

MATERIALS AND METHODS

Cell culture

The human neuroblastoma cell line CHP-100 was kindly provided by Dr. Audrey Wilson and Al Wilson from the Children's Hospital of Philadelphia. Cells were maintained in culture at 37°C in a 5% CO_2 incubator in RPMI 1640 medium (GIBCO, Paisley, United Kingdom) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin.

Cell volume measurement

Two techniques were utilized to measure changes in cell volume. The first technique involved a Coulter Multisizer (Coulter Electronics Limited, Luton, Beds, United Kingdom) coupled to a multichannel analyzer, as described by Olson et al. (1986). Cells were suspended initially in RPMI 1640 medium sized prior to exposure to ouabain, and then resized. The Coulter Multisizer Accucomp 1.15 (Coulter Electronics Limited) computer software was used for data acquisition and analysis.

Four-dimensional confocal microscopy techniques, as described by Fricker and White (1992) and White (1995) were also utilized to investigate changes in cellular volume. Briefly, cells grown on coverslips were labeled with 4.3 μM chloromethyl-fluorescein diacetate (CMFDA; Molecular Probes, OR) for 20 min at 35°C. After 3 washes with isoosmotic buffer (see below), an initial three-dimensional (3D) image was acquired, and the cells were subsequently exposed to hypoosmotic buffer (see below) and an image captured at 0.5, 1.5, 2.5, 3.5, 5, 10, 12, 15, and 20 min after treatment. Data were corrected for geometric distortions incurred by our collection conditions (White, 1995). A Nikon ×60, 1.4 N.A. oil-immersion objective lens with the pinhole fully closed was utilized. 3D intensity reconstructions were made at each time point. The brightness represented fluorescence intensity, and color was used to code relative volume with respect to time point zero. This was achieved by extracting cell volume and then generating the 3D reconstruction. All image data and manipulation were performed by using the software packages COMOS and MPL (Bio-Rad Microscience Ltd., Hemel-Hempstead, United Kingdom).

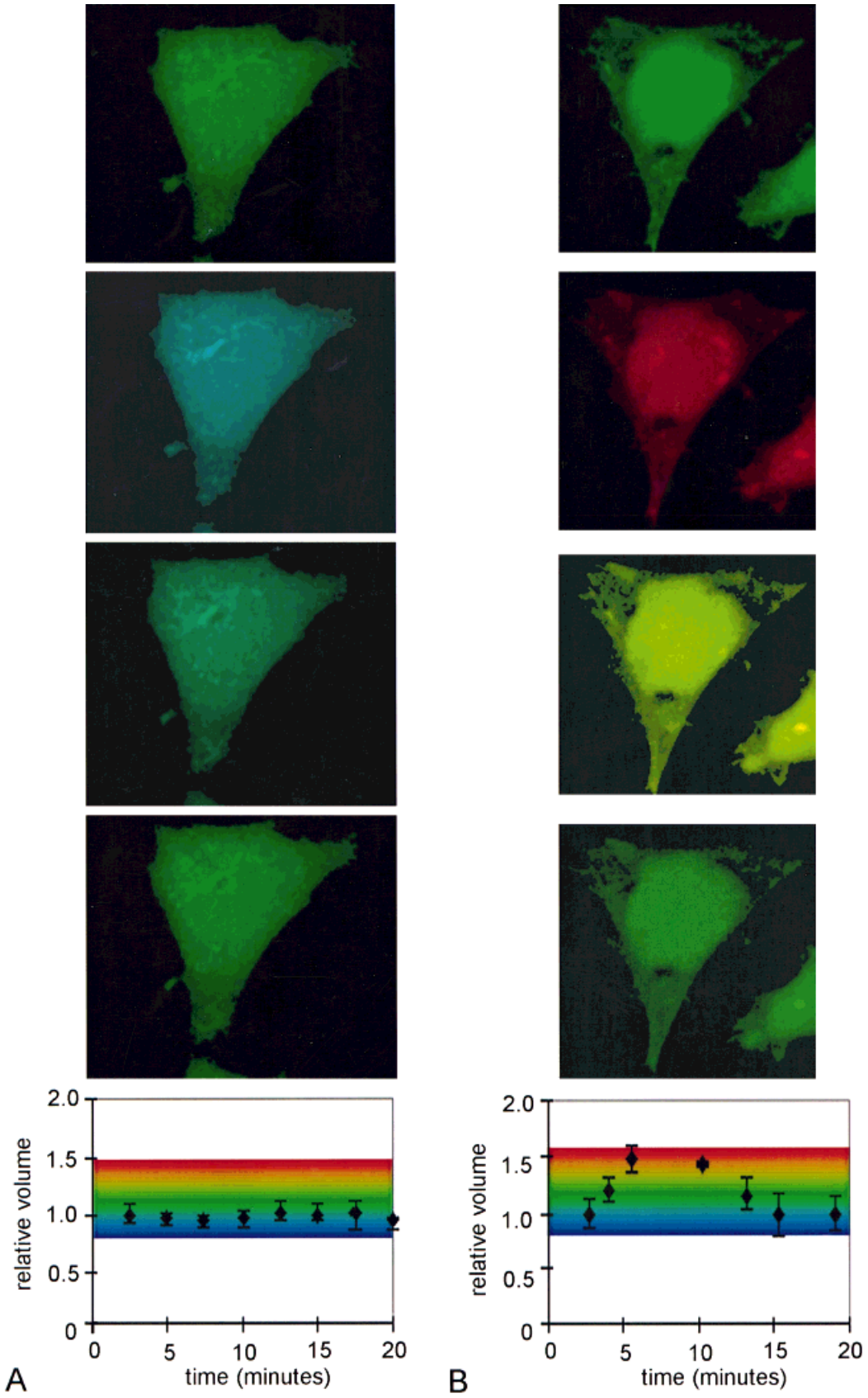
Western blotting

CHP-100 cells were lysed with Laemmli sample buffer (Laemmli, 1970) (Sigma, Poole, United Kingdom) and the lysate heated to 95°C for 3 min prior to loading on to a 7.5% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) minigel (Bio-Rad, Hemel Hempstead, United Kingdom). Following electrophoresis, the separated polypeptides were transferred to Hybond ECL nitrocellulose membrane (Amersham, Little Chalfont, United Kingdom), as described by Towbin et al. (1979). The blots were blocked in Blotto-Tween buffer (Sigma; see below) overnight with gentle agitation and subsequently incubated with recommended dilutions (approximately 1:1,000) of the primary monoclonal antibodies against the catalytic α subunits of Na^+, K^+-ATPase at 4°C overnight. The following antibodies for the specific isoforms of the α subunit of Na^+, K^+-ATPase were used: 6F, monoclonal (mouse) specific for the α1 isoform (gift from Dr. D. Fambrough, Johns Hopkins University, Baltimore, MD); McB2, monoclonal (mouse) specific for the α2 isoform (gift from Dr. K. Sweadner, Harvard Medical School, Boston, MA); and MA3-915, monoclonal (mouse) specific for the α3 isoform (Affinity BioReagents, Neshanic Station, NJ). Unbound antibody was removed by three sequential 10-min washes at ambient temperature with Tris-buffered saline (TBS). Membranes were subsequently incubated for 60 min at ambient temperature with gentle agitation in alkaline phosphatase (AP)-conjugated antimouse Ig second antibody (Sigma) diluted in TBS. Unbound antibody was removed by four sequential 10-min washes with TBS. Membranes were probed with AP-conjugated second antibody and subsequently developed and visualized by using premixed Nitroblue Tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in TBS as substrate (Knecht and Diamond, 1984).

Immunofluorescence

CHP-100 cells grown on glass coverslips were fixed for 10 min in 3.7% paraformaldehyde in PBS solution. The fixed cells were permeabilized for 5 min in 0.2% Triton X-100 in PBS and washed three times for 20 min each in PBS. Nonspecific binding was blocked by overnight incubation with 10% normal goat serum in PBS (Amersham). Cells were subsequently centrifuged and resuspended in media containing primary anti-Na^+, K^+-ATPase α subunit antibodies (α1, α2, and α3) and 1% normal goat serum. After overnight incubation at 4°C, unbound primary antibody was removed with three washes in PBS and incubated for 2 hr with fluorescein isothiocyanate (FITC; Sigma)-conjugated antimouse Ig (Sigma) diluted in PBS (1:64 titer). The cells were subsequently washed three times and mounted in Citifluor (glycerol-phenylenediamine) on microscope slides to eliminate fluorescent fading. Immunofluorescence images were subsequently taken by using a Leica-Leitz microscope fitted with a ×100 oil-immersion lens and FITC filter. Images were developed and scanned for digital analysis by using a 35 mm Nikon CoolScan LS-100 slide scanner operated by an Apple Power Macintosh. The images were analyzed by using the public domain software NIH Image for Macintosh version 1.59 (National Institutes of Health, Bethesda, MD).

Fig. 1. Effects of hypoosmotic stress on cell volume in adherent cells. Confocal microscopy techniques were used to measure changes in cell volume in adherent cells as described in Materials and Methods (reconstructions were made for each image to generate views illustrated in Panels A and B). **A:** Under isoosmotic conditions, cell volume did not change significantly, as indicated by the maintenance of the green shade. **B:** Exposure to hypoosmotic solution rapidly increased cell volume (indicated by change in color from green to red) to be followed by regulatory volume decrease (RVD) (red to green color). The bottom insets graphically represent the relative change in cell volume under isoosmotic (A) and hypoosmotic (B) conditions.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A
relative volume
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Figure 1.

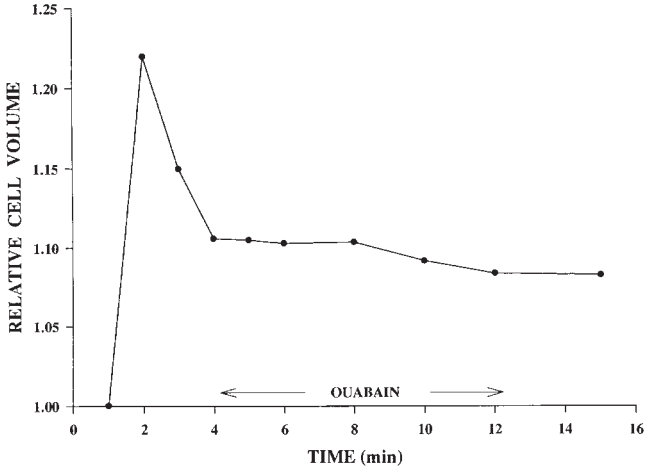

Fig. 2. Effects of blocking Na^+, K^+-ATPase on cell volume. Utilizing the Coulter Multisizer, changes in cell volume were measured subsequent to ouabain treatment (30 μM). In this representative study, cells continuously exposed (from 1 to 15 min) to ouabain resulted in rapid cell swelling followed by RVD. Results are expressed as change in relative cell volume compared with preouabain treated conditions.

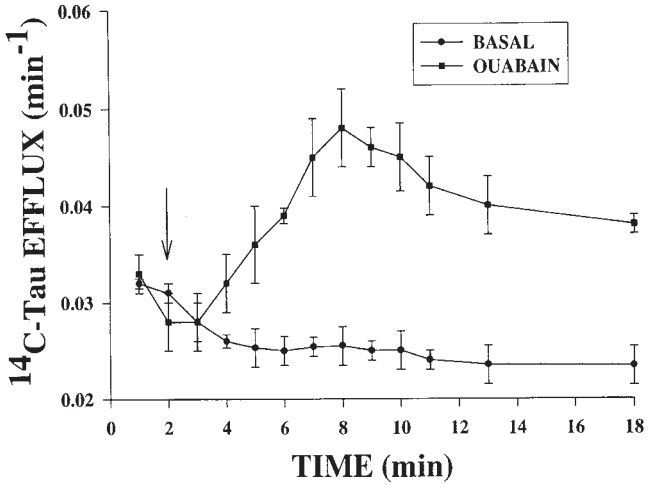

Fig. 3. Swelling-activated taurine efflux. In cells loaded with ^{14}C-taurine, exposure to 30 μM ouabain (at arrow) significantly increased taurine efflux (squares) compared with untreated cells (circles; n = 3).

Na^+, K^+-ATPase density

^{3}H-ouabain binding was used to assess Na^+, K^+-ATPase density (Erdmann, 1982), in a K^+-free medium. Cells were equilibrated for 2 hr with 1 μCi/ml ^{3}H-ouabain in DMEM media without K^+, amino acids, or vitamins. The cells were then washed in ice-cold, unlabelled K^+-free medium prior to lysing with 0.5% v:v Triton X-100 for scintillation counting. Nonspecific binding was determined by equilibrating the cells in the above media with 10^{-4} ouabain.

Taurine efflux

Cells grown to 75–90% confluence on 22 mm plates (CoStar) were incubated at 37°C with supplemented RPMI 1640 media containing ^{14}C-taurine (0.1 μCi/ml). After 1 hr, cells were washed three times with isotonic media (see below), and efflux was measured by adding and removing 1 ml of media at 1-min intervals. After a 4-min period to establish basal efflux rate, medium containing ouabain (10, 30, or 50 μM) or hypoosmotic solution (see below) plus ouabain was added. At the end of the study, the cells were lysed with 0.1N NaOH to determine the amount of radioisotope remaining inside the cells. Samples were counted in a β-scintillation counter. Efflux rate at each time point was expressed as a ratio of counts at that time point divided by the total remaining counts (Venglarik et al., 1990; Basavappa et al., 1993). For comparative studies, data are presented as percent increase in peak efflux (([peak efflux-basal efflux]/basal efflux) × 100).

Solutions and reagents

The isotonic medium contained (in mM) 140 NaCl, 4 KCl, 1 KH_2PO_4, 2 $MgCl_2$, 1.5 $CaCl_2$, 3 glucose, and 10 HEPES, pH 7.4. The final hypoosmotic solution contained (in mM) 93 NaCl, 4 KCl, 1 KH_2PO_4, 2 $MgCl_2$, 1.5 $CaCl_2$, 3 glucose, and 10 HEPES, pH 7.4. The osmolalities for the isotonic and hypoosmotic solution, as measured by freezing-point depression osmometer (Camlab Limited, Cambridge, United Kingdom), were 290 mOsm/kg H_2O and 190 mOsm/kg H_2O, respectively. For Western Blot studies, the Blotto Tween buffer contained (in mM) 5% w:v Marvel and 0.05% w:v Tween-20 in TBS, 20 Tris, and 137 NaCl, pH 7.4. Unless specified, all reagents were from Sigma.

Data analysis

Results are presented as mean ± SEM, where *n* refers to number of monolayers for efflux studies, cell suspensions for Coulter Multisizer, and number of cells for confocal microscopy. Statistical comparisons were by Student's *t* test with a significance level of $P < 0.05$.

RESULTS

Cell volume

Cell volume regulation has been investigated in both attached and suspended cells. However, differences may exist in the response to hypoosmotic conditions between attached and suspended cells (Ikehara et al., 1992). Our previous measurements of cellular volume were performed with CHP-100 cells in suspension (Basavappa et al., 1996). To determine whether a similar pattern of cell swelling and RVD occurs in adherent CHP-100 cells, confocal microscopy measurements were employed. The mean volume of ten cells was 3,547 ± 1,215 μm^3. Although, some variations in cell shape and, hence, basal cell volume were observed, the majority of neuroblastoma cells were triangular. Thus, for subsequent RVD experiments, only triangular-shaped cells were utilized with confocal microscopy.

3D images were obtained from the same cell after reducing the osmolarity of the extracellular solution from 290 to 190 mOsm/kg H_2O. Figure 1B shows the results of a typical time course of events. The montage shows changes in relative volume (Fig. 1, graph) and corresponding reconstructions of four key time points. This visualization procedure indicates relative changes in cell volume (Fig. 1, coded in color) with respect to initial volume at time point zero (Fig. 1, green). Under

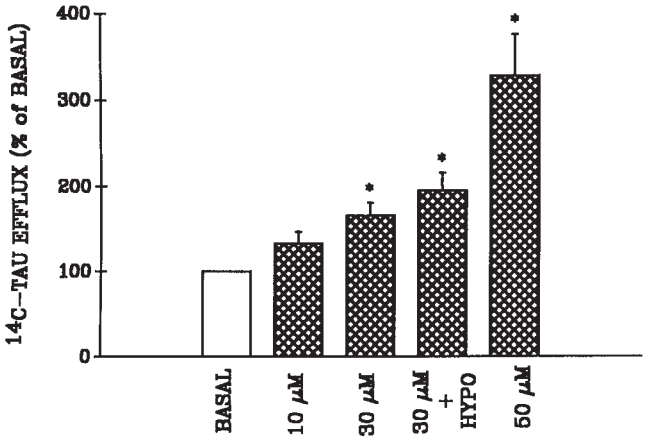

Fig. 4. Effects of ouabain on taurine efflux. Cells exposed to 30 μM ouabain and/or hypoosmotic stress (by decreasing the NaCl concentration form 140 mM to 93 mM) showed a significant taurine efflux (however, the effects were not additive). A significantly greater efflux was observed with 50 μM ouabain (compared with 30 μM ouabain). In contrast, CHP-100 cells treated with 10 μM ouabain did not increase taurine efflux significantly (n = 3–6). Asterisks, $P < 0.05$, with respect to control cells.

isoosmotic conditions, the mean change in relative cell volume was ±3% (Fig. 1A). Subsequent to the addition of hypoosmotic solution, the cell swelled by 47% (e.g., changed from green to red). The cell subsequently exhibited RVD (Fig. 1, red toward green), achieving close to 100% recovery after 9 min. Additional time points were collected to ensure that the cells remained viable after accomplishing volume recovery. Combining the three time series (n = 3), the mean volume increase (mean ± S.D.) was 37 ± 15%, and the mean rate of recovery was 4.2 ± 1.1% min^{-1} to a restored relative volume of 97 ± 16%.

The effects inhibiting Na^+-K^+-ATPase on cell volume were investigated with the Coulter Multisizer. CHP-100 cells exposed to the Na^+-K^+-ATPase inhibitor ouabain (30 μM) increased in cell size by 22 ± 4% (n = 5) within 1 min of exposure (Fig. 2). This was followed immediately by an RVD response as the cell volume gradually approached isoosmotic levels over a 15-min time period (Fig. 2). However, the cell did not completely obtain isoosmotic cell volume.

Taurine efflux

Previous studies have indicated that hypoosmotic stress activates taurine efflux in CHP-100 cells (Basavappa et al., 1996). To determine whether similar effects are observed with ouabain, cells were loaded with ^{14}C-taurine for 1 hr, and efflux of taurine was measured. Exposure to 30 μM ouabain increased taurine efflux by 183 ± 11% (n = 6; $P < 0.05$) compared with control cells (Fig. 3). In cells exposed simultaneously to 30 μM ouabain and hypoosmotic solution (decreasing the extracellular NaCl concentration from 140 mM to 93 mM), taurine efflux increased to 207 ± 18% (n = 6; $P < 0.05$; Fig. 4). At a lower concentration of ouabain (10 μM), no significant increase taurine efflux ($P > 0.05$; Fig. 4) was observed. However, 50 μM ouabain increased taurine efflux to 328 ± 68% (n = 3; $P < 0.05$; Fig. 4). Blebbing in the cell processes was observed in some cells (three of five) exposed to 50 μM ouabain under ×60 microscopy, which was not resolved after >15 min (the time normally required for RVD to be completed). Because the cell is unable to complete RVD in the presence of cellular blebbing, the observed increase in taurine efflux with greater concentration of ouabain may be indicative of a compensatory mechanism by the cell to assist in decreasing cellular volume. Similar inhibition of RVD in the presence of membrane blebs has been described in N1E115 neuroblastoma cells (Lippman et al., 1995).

Na^+, K^+-ATPase isoform expression

Expression of the three different catalytic α isoforms of Na^+, K^+-ATPase were evaluated with isoform specific monoclonal antibodies. Western blot studies showed that two α isoforms (α1 and α3) are expressed in the neuroblastoma cell line CHP-100 (Fig. 5). The most abundant isoforms were the ubiquitously expressed "housekeeping" α1 and the neuronal specific α3, with α1 expression being slightly greater than α3 (Fig. 5). In contrast, the α2 isoform was undetectable. Similar results were obtained with monoclonal antibody immunofluorescence assays, with a high level expression of α1 and α3 isoforms (Fig. 6). In addition, large pools of the α1 isoform were detected in juxtanuclear nuclear stores. In contrast, the expression of the α3 isoform appeared to be confined the plasma membrane.

Pump density

The abundance of Na^+, K^+-ATPase pump units at the plasma membrane was quantified in CHP-100 cells with ^{3}H-ouabain binding. Following a 2-hr incubation period at 37°C in K^+-free conditions, equilibrium binding was achieved. Maximal ouabain binding indicates an average of 2.83 ± 0.28 × 10^5 (n = 3; Fig. 7) pump sites/cell.

DISCUSSION

Cerebral ischemia, one of the consequences of abnormal neuronal swelling, can activate a cascade of events leading to permanent brain damage. In ischemic cells, intracellular levels of ATP are reduced, resulting in decreased Na^+ pump activity and eventual cell death. The present studies examined ouabain as a model for inducing ischemic conditions in the human neuroblastoma cell line CHP-100. Our results indicate that blocking Na^+, K^+-ATPase activity with ouabain results in an increased cell volume and an associated increase in taurine efflux to reduce neuronal swelling.

Our previous studies in CHP-100 cells demonstrated that decreasing the osmolarity from 290 mOsm/kg H_2O to 190 mOsm/kg H_2O resulted in rapid cell swelling followed by RVD (Basavappa et al., 1996). However, these cells were exposed to trypsin prior to volume measurements in suspended cells. Trypsin may disrupt key membrane properties and yield inaccurate results. In addition, Ikehara et al. (1992) report different patterns of cell volume regulation in hypoosmotic media between attached and suspended HeLa cells. To counteract the above effects, confocal microscopy techniques were utilized with cells grown in monolayer. Furthermore, Raat et al. (1996) concluded that the confocal microscopy technique maybe more reliable in measuring cell vol-

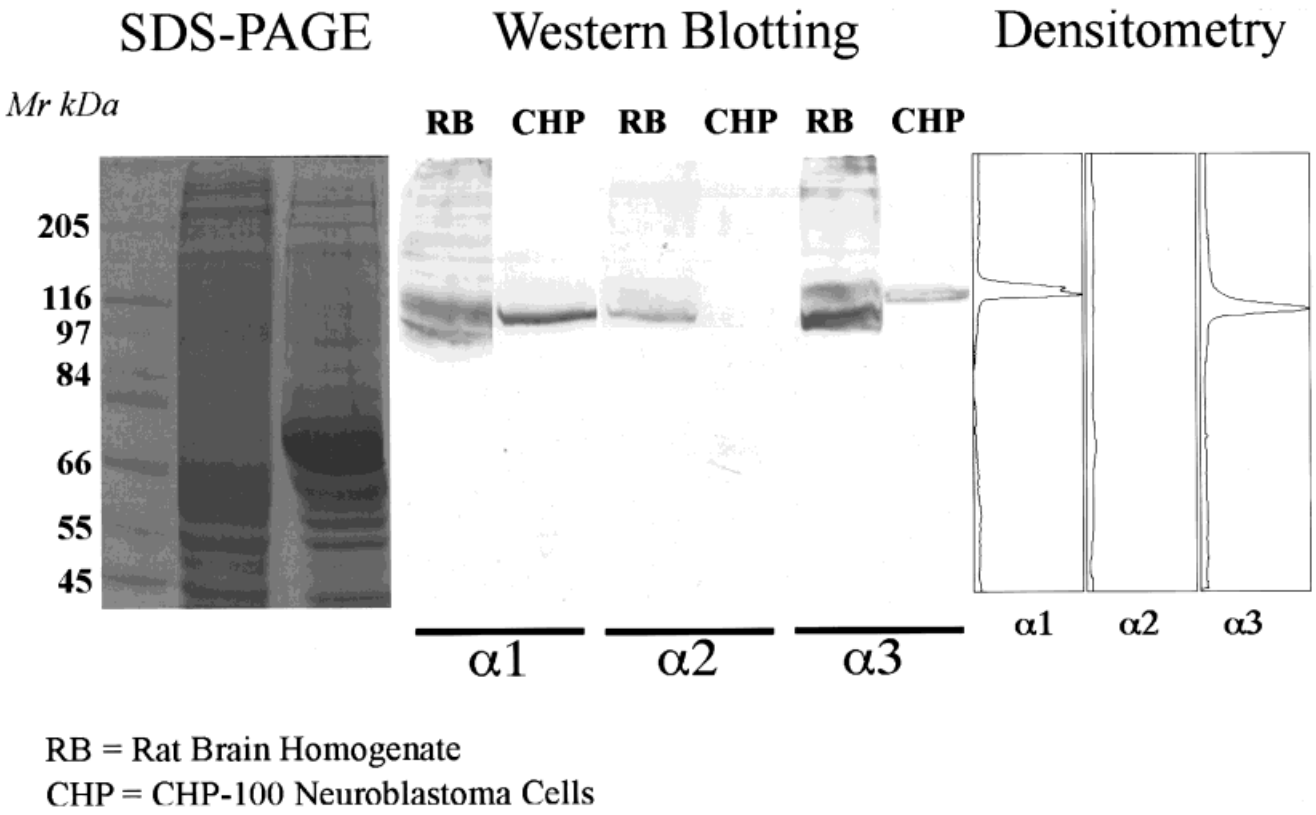

Fig. 5. The catalytic isoforms of Na^+, K^+-ATPase. By using Western blot techniques with α-isoform-specific monoclonal antibodies, the two α isoforms were identified. High levels of the ubiquitous α1 isoform and the neuronal α3 isoform were expressed. SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

ume changes in single cells compared with other methods currently available. The present studies utilizing confocal microscopy exhibited results similar to those described for cells in suspension. The initial cell swelling was followed by a rapid RVD response. Similar changes in cellular volume were observed by using confocal microscopy techniques in N1E115 neuroblastoma cells (Lippman et al., 1995).

Blocking Na^+, K^+-ATPase with the cardiac glycoside ouabain induced a rapid swelling in CHP-100 cells prior to RVD. In the glial cell line ROC-1 (Jurkowitz-Alexander et al., 1992), in rabbit cortical collecting tubule principal cells (Strange, 1991), and, recently, in N1E115 neuroblastoma cells (Lippmann et al., 1995), blockade of the Na^+ pump with ouabain has been shown to induce rapid cell swelling followed by RVD. Similar cell swelling and RVD with ouabain were observed in CHP-100 cells. By contrast, in *Helix aspersa* neurons, the most common effect observed with ouabain treatment was cell shrinkage (Alvarez-Leefmans et al., 1992). However, in a limited number of neurons, cell swelling followed by RVD was observed. The authors concluded that cell shrinkage by ouabain was a result of increased Ca^{2+}-activated K^+ efflux, leading to loss of osmolytes and water (Alvarez-Leefmans et al., 1994).

The amino acid taurine is found in high concentration in neuronal cells, where it may behave as an osmoregulator (Huxtable, 1989). During RVD, efflux of taurine has been demonstrated in many cell types, including brain cells, such as, Müller cells (Faff-Michalak et al., 1994), cerebellar granular cells (Schousboe et al., 1991), astrocytes (Pasantes-Morales and Schousboe, 1988; Kimelberg et al., 1990; Jackson and Strange, 1993), piriform cortex of rats (Wade et al., 1988), and, most recently, in CHP-100 neuroblastoma cells (Basavappa et al., 1996). In the above studies, the extracellular environment was modified to generate a hypotonic solution. However, in the present study, cell swelling was induced by blocking the Na^+ pump and, subsequently, intracellular ionic strength. The resultant increase in swelling-induced taurine efflux suggests that the mode of cell swelling is not a crucial determinant for RVD and further confirms the important role of taurine during volume regulation in CHP-100 cells.

Simultaneous exposure to ouabain and hypoosmotic solution resulted in an increased taurine efflux of ~207%, which is significantly lower than the 300% increase in swelling-induced taurine efflux previously observed under hypoosmotic conditions alone (Basavappa et al., 1996). This may suggest that increased intracellular ionic strength (resulting from blockade of Na^+, K^+-ATPase) may modulate the swelling-induced activation of taurine efflux. This is consistent with the observations by Motais et al. (1991) in trout erythrocytes, in which the swelling-induced taurine efflux was

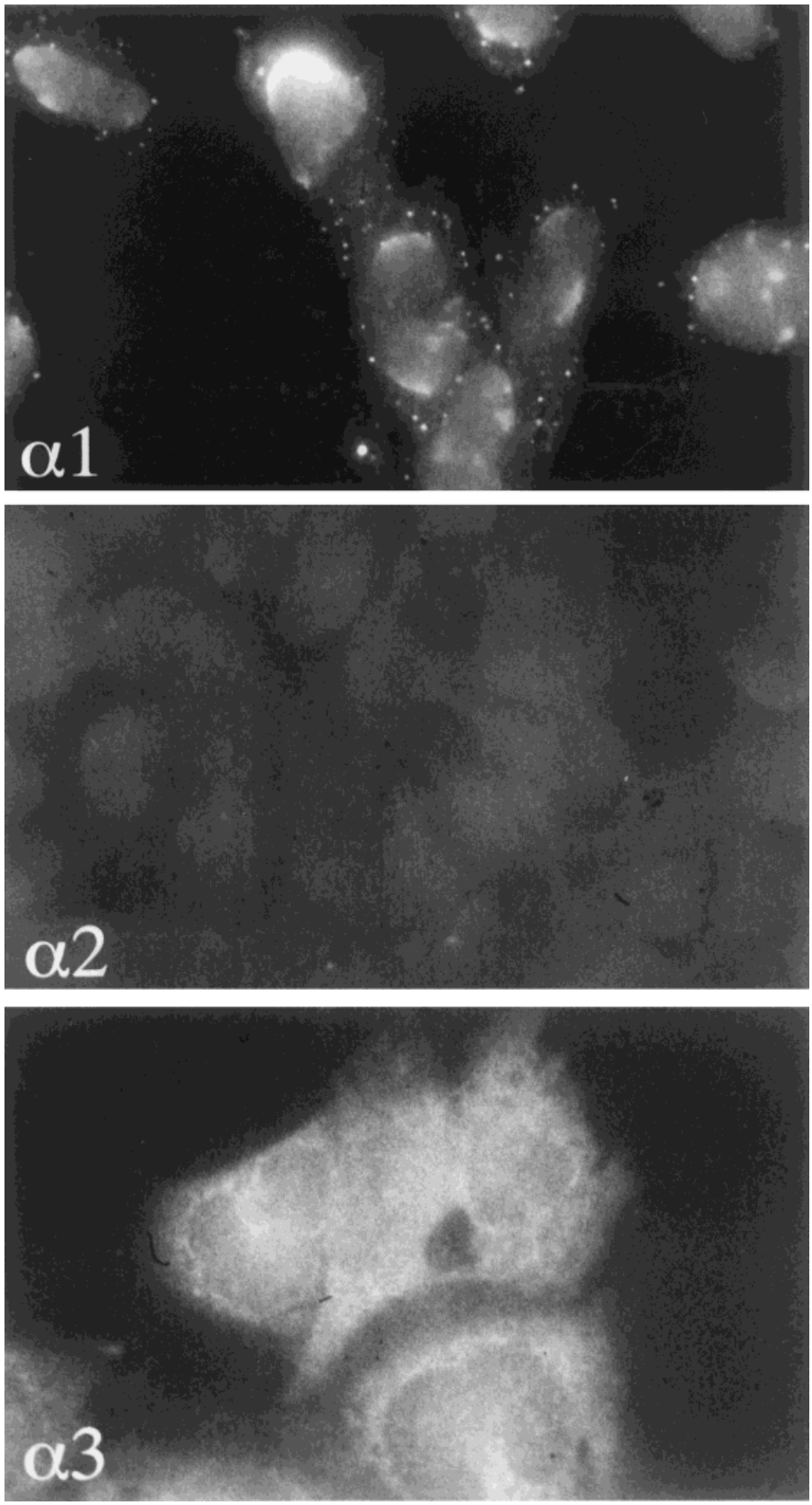

Fig. 6. Detection of Na^+, K^+-ATPase α isoforms by immunofluorescence. The α1 isoform (**top**) was detected in CHP-100 cells fixed in paraformaldehyde and probed with a monoclonal antibody specific for the α1 isoform (*6F*). Similarly, the α3 isoform was observed (**bottom**) with cells treated with the α3-specific monoclonal antibody (*MA3-915*). However, the α2 isoform was not detected (**middle**) in cells exposed to the α2-specific monoclonal antibody (*McB2*).

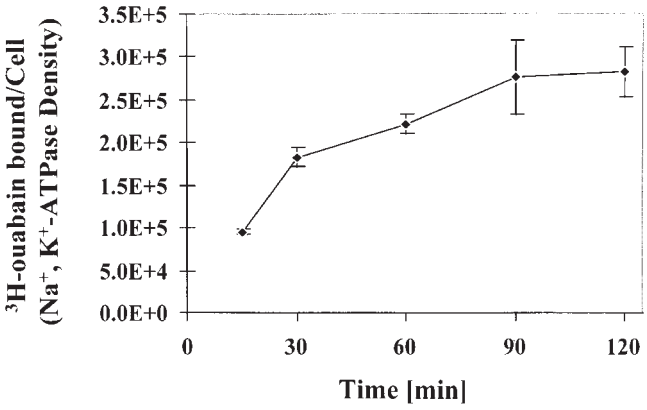

Fig. 7. Na^+, K^+-ATPase pump density. The density of Na^+, K^+-ATPase pumps at the plasma membrane were quantified with ^{3}H-ouabain binding, as described in Materials and Methods. CHP-100 cells were found to express $\sim 3 \times 10^5$ pump units/cell (n = 3).

inhibited by increased intracellular NH_4Cl concentration. The authors suggest that intracellular ionic strength plays a key role in regulating swelling-induced amino acid efflux. Similarly, recent studies in skate hepatocytes by Jackson et al. (1996), demonstrate inhibition of swelling-activated anion conductance with elevated cytosolic anion concentration. Taken together, the above studies strongly favor regulation of the swelling-induced taurine efflux by intracellular ionic strength. However, the observed differences in taurine efflux in CHP-100 cells also may be attributed to differences in cell swelling. Additional studies are clearly indicated to elucidate the possible role of intracellular ion strength during RVD.

Preliminary studies in CHP-100 cells indicate that blockade of Na^+-K^+-ATPase increases cytosolic Ca^{2+} levels via a route sensitive to removal of extracellular calcium with EGTA (Basavappa et al., unpublished results). Similar effects with ouabain were observed in *Helix aspersa* neurons (Alvarez-Leemans et al., 1994) and in sensory neurons of the leech central nervous system (Schlue, 1991). In CHP-100 cells, we have previously shown that hypoosmotic stress increases cytosolic Ca^{2+} levels via voltage-sensitive P-type Ca^{2+} channels. Following calcium entry, swelling-induced Cl^- conductance pathways are stimulated (Basavappa et al., 1995). Similarly, hypoosmotically activated taurine efflux is dependent upon Ca^{2+} influx in CHP-100 cells (Basavappa et al., 1996).

The plasma membrane of Na^+, K^+-ATPase consists of a catalytic α subunit (110–112 kDa) and a regulatory β subunit (35 kDa; Sweadner, 1989). To date, four α and two β subunit isoforms have been identified in mammals, each encoded by a separate gene and exhibiting a tissue-specific pattern of expression (Shyjan et al., 1990; Takeyasu et al., 1990; Shamraj and Lingrel, 1994; Sweadner, 1995). Characteristically, the α1 isoform is ubiquitously expressed, whereas the α2 isoform has been detected in brain and muscle, α3 is found predominantly in neuronal tissue, and α4 is found exclusively in the testis (Shyjan et al., 1990). Three α isoforms of Na^+-K^+-ATPase have been detected in the central nervous system (Urayama et al., 1989), but, to our knowledge, all three isoforms have not been detected in one neuronal cell type. Recent observations suggest that α3 is expressed mainly in neuronal cells, α2 is expressed in glial cells, whereas α1 is expressed in both cell types (Sweadner, 1995). However, not all neurons express α3, and not all glial cells express α2 (Sweadner, 1995). For example, α1 and α3 have been detected in retinal ganglion cells, amacrine cells, and horizontal cells (McGrail and Sweadner, 1986); in hippocampal pyramidal cells (Brines et al., 1991; McGrail et al., 1991; Pietrini et al., 1992); and in subpopulations of dorsal root ganglion cells (Mata et al., 1991). Similar expression of α1 and α3 isoforms of the catalytic subunit of the Na^+, K^+-ATPase was detected in CHP-100 cells. Although HeLa cells endogenously express only α1, Jewell and Lingrel (1991) were able to express all three α isoforms by transfection in HeLa cells with rat Na^+, K^+-ATPase α1, α2, and α3 isoforms. The relative expression of the α isoforms in CHP-100 cells was $\alpha 1 > \alpha 3$.

Thus, in the human neuroblastoma cell line CHP-100, the following sequence of events may occur subsequent to blockade of Na^+, K^+-ATPase by ouabain, which may mimic cerebral ischemia: 1) increased intracellular ionic strength and cell swelling; 2) membrane depolarization, which may activate voltage sensitive Ca^{2+} channels (most probably the P-type Ca^{2+} channel (Basavappa et al., 1995); and 3) calcium-activated efflux of taurine, returning the cell volume to isoosmotic levels.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. D.M. Fambrough (Johns Hopkins University, Baltimore, MD) and Dr. K.J. Sweadner (Harvard Medical School, Boston, MA) for their kind gift of monoclonal antibodies 6F and McB2, respectively. Support from the Wellcome Trust and the Arthritis and Rheumatism Council are gratefully acknowledged.

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