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Characterization of the Taurine Transport Pathway in A6 Kidney Cells

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Abstract. We investigated the role of taurine in cell homeostasis and characterized the taurine transport pathway in cultured kidney cells (A6). The taurine concentration in A6 cells varies with the osmolarity of the culture medium, suggesting that taurine participates in cell osmolarity. Under isosmotic conditions, ^{14}C-taurine efflux through the apical membranes (aJ_{taur}) was 6–7 times lower than that through the basolateral membranes (bJ_{taur}). Under hyposmotic conditions, aJ_{taur} remained almost unchanged. On the contrary, bJ_{taur} increased 8 times in comparison with isosmotic conditions. In hyposmotic conditions, bJ_{taur} was inhibited by 500 μM DIDS, 50 μM NPPB, 10 μM of the two oxonol derivatives DISBAC(2)3 and WW-791, and 100 μM ketoconazole. Conversely, 100 μM 1,9-dideoxyforskolin, 10 μM tamoxifen, 100 μM niflumic acid and 50 μM verapamil had no inhibitory effects. Cell volume regulation upon hyposmotic stress was also found to be inhibited by DISBAC(2)3 ($K_{0.5}$ of 5 ± 1 μM) and by ketoconazole. Nystatin was used to permeabilize the apical membranes with the aim to further characterize bJ_{taur}. ^{14}C-taurine transepithelial fluxes in nystatin-treated cells were found to be linear over taurine concentrations ranging from 3.5 μM to 35 mM. Clamping the transepithelial voltage at positive values (serosal side) slightly stimulated the ^{14}C-taurine transport. Similar time courses of ^{14}C-taurine, ^{36}Cl and ^{86}Rb transepithelial fluxes were found under osmotic stimulation followed by DIDS inhibition in nystatin-treated cells. In whole cell patch-clamp experiments, DISBAC(2)3 application resulted in a strong and reversible decrease of the global Cl^- current which was stimulated by hyposmotic stress. Our study indicates that taurine participates in the control of A6 cell osmolarity and that the transporting taurine pathway (efflux) is on the basolateral membranes. In addition to usual chloride channel blockers, oxonol was found to be a potent blocker of the taurine transport and of the swelling-activated chloride current. Using a pharmacological approach, we could not distinguish between a common or different pathway for Cl^- and taurine.

Key words: Epithelia — Taurine transports — Chloride channel — Oxonol

Introduction

The amphibian renal A6 cell line forms highly polarized monolayers and has been used as a model for studying Na^+ and Cl^- transport in epithelia of high resistance under hormonal regulation (Perkins & Handler, 1981; Sariban-Sorhaby, Burg & Turner, 1983; Chalfant, Coupaye-Gerard & Kleyman, 1993; Verrey, 1994; Banderali et al., 1999).

These cells, submitted to a hyposmotic shock, swell by rapid water flow through the basolateral membranes (blm), and subsequently regulate their volume (Ehrenfeld, Raschi & Brochiero, 1994; Crowe et al., 1995; Nilius et al., 1995; De Smet, Simaels & Van Driessche, 1995a). Regulatory volume decrease (RVD) is blocked by a number of potassium and chloride channel blockers, suggesting that the opening of both channels is involved in cell volume regulation (Ehrenfeld et al., 1994; Nilius et al., 1995). Using patch-clamp techniques, we could identify four different chloride channels stimulated under hyposmotic conditions in the blm of A6 cells (Banderali & Ehrenfeld, 1996). The increased activity of these channels under hyposmotic conditions appeared as the initial event responsible for the observed cell membrane depolarization that favors subsequent potassium exit, thereby allowing cell volume regulation (Brochiero et al., 1995).

During RVD, most cells also activate transporting pathways for various organic osmolytes (for review *see* Garcia-Perez & Burg, 1991; Burg, 1994;

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Kirk, 1997) such as polyols (e.g., sorbitol), methylamines (e.g., betaine) and amino acids (e.g., taurine). Renal cells (including cell lines) have been particularly valuable for studying these phenomena since large osmotic gradients are found between the medulla and the cortex of the kidney (Nakanishi, Uyama & Sugita, 1991; Boese, Wehner & Kinne, 1996). However, in amphibian renal cells, very few studies have concerned the characterization of amino-acid transport pathways (De Smet et al., 1995b) and to our knowledge, none have concentrated on that of taurine.

Roy and Malo (1992) were the first to suggest that effluxes of amino acids could be mediated through volume-activated anion channels. Strong evidence supporting this hypothesis was found later with different cell types including MDCK (Banderali & Roy, 1992), flounder erythrocytes (Kirk, Elloty & Young, 1992), endothelial cells (*see* Nilius et al., 2000) or C6 glioma cells (Jackson & Strange, 1995). To date, the molecular nature of the taurine-transporting pathway is still unknown. In most cell types, swelling-activated Cl^- currents and the osmolyte effluxes share many similarities, supporting the idea of a common pathway (*see* review by Okada, 1997; Kirk, 1997). The channel supposed to mediate this pathway has been termed VSOAC for volume sensitive organic osmolyte/anion channel but has also been referred to as VRAC (for volume-regulated anion channel) or VSOR (for volume-sensing outwardly rectifying Cl^- channel) in other cell types. However, in HeLa cells, distinct taurine and chloride efflux pathways activated during regulatory volume decrease were recently reported. Differences in anion sensitivity, the time course of activation and the sensitivity to DIDS were found for taurine and Cl^- efflux pathways (Stutzin et al., 1999). Different pharmacological profiles suggesting separate pathways were also reported for Ehrlich ascites tumor cells (Lambert & Hoffmann, 1994). For *Xenopus laevis* oocytes expressing anion channels, the taurine efflux was increased, whereas the chloride conductance was not, and therefore two separate pathways were also proposed (Stegen et al., 2000).

In the present study, we aimed to determine the physiological role(s) of taurine as an organic osmolyte in A6 cells. The cells were grown on permeant supports under iso-, hypo- and hyperosmotic conditions and the taurine concentration in the cells was determined. The taurine effluxes through the apical and basolateral membranes were studied under iso- and hyposmotic conditions and the hyposmotically-activated taurine transport pathway was characterized by a pharmacological approach. To determine whether the taurine and chloride transport pathways were identical in A6 cells, we further examined the basolateral membrane permeabilities to ^{36}Cl, ^{86}Rb and ^{14}C-taurine (after permeabilizing the apical membranes with nystatin) in response to cell swelling and subsequent exposure to DIDS.

Materials and Methods

Cell Culture

A6, a renal cell line derived from *Xenopus laevis*, was a gift of Dr Rossier (Lausanne, Switzerland). These cells had originally been obtained from the American Tissue Type Collection and were subsequently cloned (clone A6-2F3) by limiting dilution (Verrey et al., 1987). Cells were grown between passages 88–98 at 28°C in a humidified atmosphere of 5% CO_2 in air. The amphibian cell medium (AM), (Handler et al., 1979), supplemented with 10% fetal calf serum (IBF, France) and antibiotics, was changed three times weekly. The osmolarity of the AM was measured using a vapor pressure osmometer (Model 5500, Wescor, Logan UT, USA) and was found to be 247 mOsm.

For transport experiments, the cells were seeded onto transparent collagen-treated membranes (Transwell, 0.45-μm pore, Costar, MA, USA) at a seeding density of 2×10^6 per well (5 cm^2). Cell monolayers were then incubated for 5–10 days with the amphibian medium (serum-free) supplemented with 2% ultroser-G (Gibco-IBF, USA-France) in order to increase their Na^+ transport capacity. The osmolarity of this solution was 247 mOsm.

Taurine Concentration and Volume Determination in A6 Cells

Confluent A6 cells (grown on plastic dishes of 78 cm^2) were adapted for 5–6 days to culture media (containing 1 mM taurine) of different osmolarities as indicated below. The cells were then loaded with ^{14}C-taurine (0.02 μCi/ml) for 48 hours. This time was found to be sufficient to reach the ^{14}C-taurine equilibrium between the cells and the medium (similar specific radioactivities). After three rapid washes with non-radioactive culture medium (to avoid extracellular ^{14}C contamination) A6 cells were scraped and lysed in 5 ml H_2O. 50-μl aliquots were withdrawn to determine protein concentration with a protein assay (Bio-Rad, Munich, Germany). The remaining solution was placed in vials supplemented with 7 ml of ACS (Amersham, France) for counting in a liquid scintillation counter (Packard Instruments, USA). Results were expressed as μmoles of taurine/mg of protein.

For volume determination, cells were adapted for five days in media of different osmolarities. After trypsination (0.05% trypsin), cells were incubated for 15 min with ^3H-inulin (0.29 μCi/ml), while keeping the osmolarity of the medium similar to the adaptation medium at 28°C in a humidified atmosphere of 5% CO_2 in air. The cells were then centrifuged in cytocrit tubes of 1.38 mm internal diameter for 60 sec at 6,000 × g in a rapid microcentrifuge (Hettich, Germany). The supernatant was drawn off and kept for counting. The length of the cell column was measured and the volume of the pellet calculated. The extracellular space in the pellet was calculated from the amount of the ^3H-inulin sedimenting between the cells. The cell volume was calculated as the difference between the pellet volume and the extracellular space. For each sample, the number of cells and the total protein concentrations were also determined. Quadruplicate samples were simultaneously processed for each cell-volume measurement. This procedure of cell-volume determination has already been used in a previous study (Ehrenfeld & Cousin, 1982) and was found to agree with that of direct cell-volume measurement by video imaging. In addition, the regulatory volume decrease in A6 cells was similar using this technique, the Coulter

counter technique and the cell height measurement in intact epithelium (*unpublished data*).

The cellular taurine concentration was calculated from the relationship: $SR_{medium} = SR_{cell}$, with SR_{medium} and SR_{cell} being the specific radioactivities of the external culture medium and of the cytosol, respectively. SR is the ratio of ^{14}C-taurine concentration in $dpm.ml^{-1}$/taurine concentration in $\mu mol.ml^{-1}$. The taurine concentration was expressed in mmol/l of cells.

The isosmotic amphibian medium (AM, 247 mOsm), the hyposmotic medium (AM diluted 1/3, 165 mOsm) and the hyperosmotic medium (AM + 40 mM NaCl, 327 mOsm) were the different adaptation media used.

^{14}C-Taurine Effluxes Through Apical and Basolateral Membranes

The cells were loaded for two hours with ^{14}C-taurine (0.15 μCi/ml) in the culture medium and then washed three times with a Ringer solution. The ^{14}C-taurine effluxes through both cell membranes were followed as a function of time by replacement of the apical and basolateral solutions (*see* below for Ringer solution composition). At the end of the experiments, the cells were incubated overnight with 1N NaOH. The collected samples were placed in counting vials supplemented with 5 ml of ACS (Amersham, France) for counting in a liquid scintillation counter (Packard Instruments, USA). The apical (aJ_{taur}) and the basolateral (bJ_{taur}) taurine effluxes were expressed as the percent of total cell radioactivity released during the 20-min efflux period.

^{14}C-Taurine, ^{86}Rb- and ^{36}Cl-Transepithelial Fluxes in Nystatin-Treated Cells

Nystatin (50 μM) was added to the apical solution in order to permeabilize the apical membranes of the A6 cell monolayers. In these experiments, ^{14}C-taurine (0.115 μCi/ml, NEN, Boston, USA), ^{86}Rb (RbCl salt, 1.5 μCi/ml, Amersham, France) or ^{36}Cl (NaCl salt, 1.5 μCi/ml, ICN, Belgium) was added to the apical bathing solution and the transepithelial (m/sec) fluxes reflecting the basolateral effluxes, were measured by simple transfer of the filter supporting the monolayer from one well to another of the 6-well cluster (Costar), each of which contained 1 ml of a Ringer solution. The radioactivity of the collected samples (i.e., the basolateral incubation medium) and in 20 μl of the apical solution were then determined as described above. ^{86}Rb and ^{36}Cl transepithelial fluxes were expressed in $\mu mole/hr.cm^2$ and ^{14}C-taurine fluxes were expressed in $pmol/hr.cm^2$. All experiments were performed at 28°C in a humidified atmosphere of 5% CO_2 in air.

Voltage-Clamp Experiments

Electrical measurements (transepithelial potential, short circuit current and resistance of monolayers) were carried out in a home-made modified Ussing chamber designed to fit the Transwell. The volumes of the apical and basolateral bathing solutions were 2 ml and 2.5 ml, respectively, and the solutions could be changed without interruption of the electrical measurements. The spontaneous transepithelial potential (PD) was measured through Agar-KCl salt bridges and was clamped at zero volts, through platinum electrodes, using an automatic voltage clamp (Model VC 600, Physiological Instrument, Houston, TX, USA). The short-circuit current (SCC) and additional pulses (10 mV, 1 sec duration every 60 sec) for measurements of the monolayer resistance (R) were continuously recorded on a chart paper recorder (SEFRAM, France).

Cell-Volume Measurement

A Coulter counter was used to measure the volume of isolated cells in suspension. This technique has been successfully used with a number of epithelial and nonepithelial (dissociated) cells, including MDCK cells, another kidney cell line, (Roy & Sauvé, 1987) and A6 cells (Ehrenfeld et al., 1994). The cell size distribution and the average volume of a finite number of cells were measured with a Coulter counter multisizer II (256 channels) connected to a PC-type computer. A program developped by Coulter counter was used to evaluate the average cell volume and the total volume of the cell population counted. Volume changes were presented as % of the initial volume measured in an isosmotic medium.

Patch-Clamp Experiments

Confluent A6 cells were first trypsinated, centrifuged and resuspended in culture medium and then plated at a density of 250,000 cells/ml in 30-mm diameter Petri dishes. Cells were kept at 28°C. Patch-clamp experiments were performed 1 to 6 hours following cell plating. Before starting the experiments, culture medium was rapidly exchanged with isotonic solution of the following composition (mM): NMDG-Cl^-, 60; $CaCl_2$, 1; $MgCl_2$, 2; HEPES, 10; KCl, 4.7; TEA-Cl, 10; mannitol, 75; (240 mOsm/l); pH 7.2. The hyposmotic solution (165 mOsmol/1) used in some experiments had the same composition, but mannitol was omitted. Oxonol (DIS-BAC2(3)) was added to the hyposmotic solution in some cases. Oxonol was first solubilized in DMSO (10^{-2} M) and then diluted to the final concentration of 10^{-5} M in the hyposmotic solution. Petri dishes were placed under a phase contrast-inverted microscope (TMS, Nikon, Japan). Glass electrodes were pulled from borosilicate hematocrite tubes (Modulhom, Denmark) using a vertical puller (PP 830, Narishige, Japan) in order to achieve resistance values ranging between 4 and 5 MΩ after back-filling with intra-pipette solution. In all experiments, intra-pipette solution was (mM): CsCl, 100; $MgCl_2$, 2; HEPES, 10; EGTA, 0.1; ATP, 2; (220 mOsm/l); pH 7.2 (adjusted with CsOH). Tight seals (2 GQ) were obtained using a PCS 5000 Burleigh piezo micromanipulator (Burleigh, USA). Currents were recorded using a Visual Patch 500 patch-clamp amplifier (Biologic, France) coupled to an IBM-compatible PC. Signals were acquired at 500 Hz sampling frequency and filtered at 200 Hz to avoid aliasing. Currents were next analyzed with Biotools (Biologic, France) and Origin (Micrococal, USA) softwares. Amplitude of currents was measured as the mean amplitude of current recorded in the last 800 msec of each voltage pulse. Hyposmotic and oxonol-containing solutions were applied to the cells during the experiments using a home-made gravity-fed micro-application system.

Drugs and Solutions

5-Nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) was provided courtesy of Dr R. Greger, University of Freiburg (Freiburg, Germany). 4,4′-Diisothiocyanastilbene-2,2′-disulfonic acid (DIDS), (±) verapamil hydrochloride, and [Z]-1-[dimethylaminoethoxyphenyl]-1,2-diphenyl-1-butene (Tamoxifen) were from Sigma (St. Louis, MO). Niflumic acid and anthracene-9-carboxylic acid were from Aldrich Chemical Co (Beerse, Belgium). Ketoconazole was from Biomol Research Laboratories (Plymouth, PA). Bis-(1,3-diethylthiobarbituric acid trimethine oxonol (DiSBAC2(3)) and WW 791 were from Molecular Probes (Eugene, OR). Stock solutions of these agents were prepared in DMSO; Niflumic acid was a gift from UPSA laboratories.

Ringer solution (mM) was: NaCl, 50; $NaHCO_3$, 24; KC1, 2.5; $CaCl_2$, 2; $MgSO_4$, 2; Na_2HPO_4, 3.2; KH_2PO_4, 1.2; glucose, 5;

Table 1. Taurine concentration in A6 cells adapted to different medium osmolarities

Medium (mOsm)	Proteins mg/78 cm^2	Cell number N 10^6/78 cm^2	Cell volume µl/78 cm^2	Taurine content µmole/78 cm^2	Taurine concentration mmole/l cells
Isosmotic	12.4 ± 1.1	48.3 ± 5.4	77.5 ± 2.8	0.20 ± 0.03	2.6 ± 0.5
Hyposmotic	7.8 ± 0.8*	29.3 ± 3.8*	60.9 ± 1.2*	0.09 ± 0.01*	1.5 ± 0.2*
Hyperosmotic	10.6 ± 3.3	51.7 ± 2.6	78.0 ± 5.0	0.57 ± 0.14*	7.3 ± 1.3*

Cells plated on Petri dishes of 78 cm^2 were adapted for 5–6 days to isosmotic amphibian medium (AM, 247 mOsm), hyposmotic medium (AM diluted 1/3, 165 mOsm) and hyperosmotic medium (AM + 40 mM NaCl, 327 mOsm) prior to measurement of the taurine concentration ($n = 6$). *Significance $p < 0.01$ (in comparison with isosmotic medium).

mannitol, 60; HEPES, 5; and the pH 7.4 after bubbling with 5% CO_2. The osmolarity of this solution was of 247 mOsm. Mannitol was omitted to obtain the hyposmotic solution (187 mOsm).

The apical solution of the nystatin-treated cells was: Kgluconate, 70; $NaHCO_3$, 24; KC1, 30; $CaCl_2$, 0.5; $MgSO_4$, 2; KH_2PO_4, 1.2; Na_2HPO_4, 3.2; glucose, 5; mannitol, 60; HEPES, 5; and the pH 7.4 after bubbling with 5% CO_2. This solution was called "Kgluconate solution", while in the "mannitol solution", 70 mM of Kgluconate was replaced by 140 mM mannitol.

Statistics

Data variability is expressed as the standard error of the mean (SEM). The Student's *t*-test was used for estimating the significance of differences between paired mean data.

Results

Cell Taurine Concentration Increases with the Osmolarity of the Culture Medium

In a first experiment, we measured the taurine concentration within A6 cells adapted to media of different osmolarities in order to evaluate the contribution of this amino acid to the osmotic cell homeostasis. In the three experimental conditions tested, the cells formed confluent monolayers and presented an apparently usual morphology. However, a reduction of the cell number was noticed for cells grown 6 days in hyposmotic media (Table 1). Compared to isosmotic conditions, the taurine content decreased (two fold) in cells adapted to hyposmotic medium and increased (three fold) in cells adapted to hyperosmotic medium. Taking into account the cell volume, the taurine concentration was also found to increase with the osmolarity of the culture medium. A 2-fold reduction of the taurine concentration with a hyposmotic medium and a 3-fold increase with hyperosmotic medium were observed. These findings indicate that taurine is accumulated in A6 cells in response to increasing osmolarity.

Apical and Basolateral Taurine Effluxes in A6 Cells under Iso- and Hyposmotic Conditions

Next, we investigated whether taurine behaves as an organic osmolyte in A6 cells and is released by the cells during RVD. Towards this aim, A6 cells were grown on permeant membranes and the apical and basolateral taurine effluxes were measured under isosmotic conditions (247 mOsm) and under hyposmotic conditions (187 mOsm). The cells were loaded with ^{14}C-taurine and the ^{14}C-taurine efflux through the two opposing cell membranes was followed as a function of time. Results are shown in Fig. 1*A*, *B*. Large differences can be observed between the permeabilities of the apical and basolateral membranes. Under both serosal isosmotic and hyposmotic conditions, the taurine efflux across the apical membrane (aJ_{taur}) was much smaller than the efflux across the basolateral membrane (bJ_{taur}): under isosmotic conditions, aJ_{taur} was 6 to 7 times less than bJ_{taur} and under hyposmotic conditions, it was 20 to 24 times less than bJ_{taur} ($n = 16$). It can also be noticed that a hyposmotic challenge induced a large (approximately 8-fold) increase in the taurine permeability of the basolateral membranes, whereas the apical permeability was only increased 2-fold. In contrast, changes in the osmolarity of the mucosal medium did not affect taurine efflux (*data not shown*), which is probably due to the very small water permeability of the apical membranes (Wills, Millinor & Crowe, 1991; De Smet, Simaels & Van Driessche, 1995c).

Under the experimental conditions cited above, the taurine permeability of the basolateral membranes was stimulated by a change in osmolarity of 24.3%. Next, we determined the relationship between the taurine effluxes and the external osmolarity. As illustrated in Fig. 1*C*, bJ_{taur} was already significantly stimulated upon a change in the osmolarity of the serosal bathing medium of 4.0%. These experiments show that a taurine transport pathway at the basolateral membrane of A6 cells is activated under hyposmotic conditions.

Pharmacological Characterization of the Basolateral Taurine Transport Pathway in A6 Cells

To further characterize the taurine transport pathway of the basolateral membranes of A6 cell monolayers, we investigated the effects of a variety of anion Channel and anion transporter inhibitors (Fig. 2). In

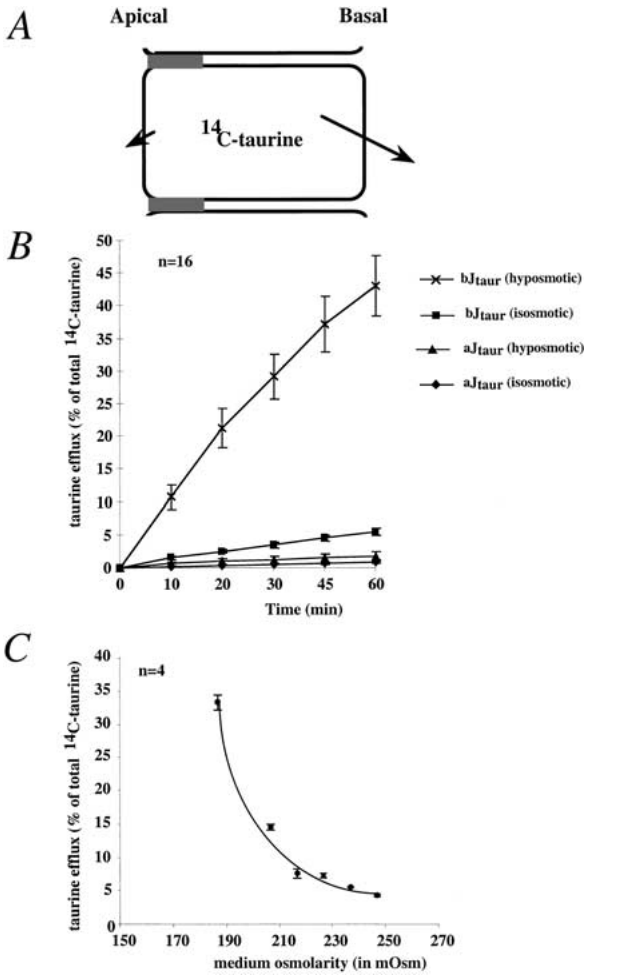

Fig. 1. Taurine effluxes in A6 cells. (*A*) Schematic representation of the ^{14}C-taurine efflux through the apical and basolateral membranes of A6 cells. (*B*) ^{14}C-taurine effluxes were measured as a function of time through the apical (aJ_{taur}) and the basolateral membranes (bJ_{taur}) under isosmotic or hyposmotic conditions (n = 16). (*C*) Basolateral ^{14}C-taurine effluxes were measured as a function of osmolarity of the medium applied to the serosal side, while the mucosal side was kept isosmotic (247 mOsm n = 4).

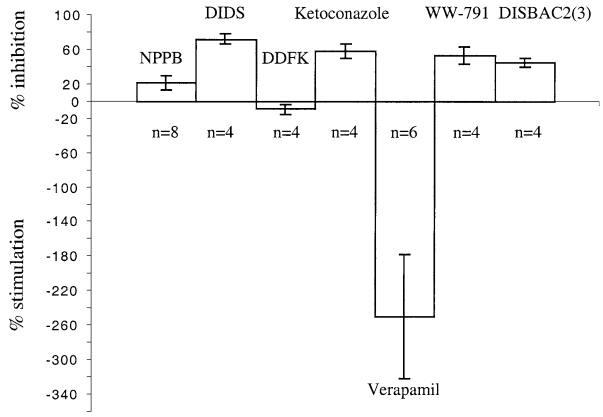

Fig. 2. Inhibitors of the basolateral taurine effluxes in A6 cells. NPPB (50 μM), DIDS (500 μM), 1,9-dideoxyforskolin (100 μM), ketoconazole (100 μM), verapamil (50 μM), WW-791 (10 μM) or DISBAC2(3) (10 μM) were added on the serosal side of the monolayer. After 15 min, the serosal bathing solution was switched to a drug-containing hyposmotic solution and ^{14}C-taurine effluxes through the basolateral membranes were measured during a 20-min period and compared to the ^{14}C-taurine efflux in the absence of inhibitors, n = number of experiments.

the following experiments, the effects of drugs were tested in cells submitted to a hyposmotic challenge (187 mOsm).

Application of NPPB and DIDS on the basal side of the cell monolayer induced a significant inhibition of bJ_{taur} (mean inhibitions after 20 min of hyposmotic shock were 32% and 78% after treatment with 50 μM NPPB and 500 μM DIDS, respectively). As in C6 glioma cells (Jackson & Strange, 1993), ketoconazole (100 μM) blocked bJ_{taur} (the mean inhibition of bJ_{taur} was 65%). Niflumic acid (100 μM) had no inhibitory effects (*data not shown*).

It has been described that verapamil and the 1,9-dideoxy derivative of forskolin inhibit the volume-activated Cl^- current associated with expression of P-glycoprotein (Valverde et al., 1992; Diaz et al., 1993). We used these agents at the same concentrations (100 μM) as in these studies. We found that 1,9-dideoxyforskolin did not affect bJ_{taur} but, surprisingly, a large (about 3-fold) stimulation of the taurine effluxes was observed after verapamil application. This stimulation was considered nonspecific, since it could not be blocked by DIDS (*data not shown*). Concentrations of verapamil of 50 μM still stimulated bJ_{taur} but doses lower than 10 μM or application of 10 μM tamoxifen (another P-glycoprotein blocker) had no significant effect (*data not shown*). These experiments indicate that P-glycoprotein is not likely to be involved in the hyposmotic-sensitive taurine effluxes in A6 cells.

Oxonol dyes have been reported to block volume-activated anion channels as well as anion exchangers (*see* Discussion). We therefore tested these agents on the taurine effluxes in A6 cells submitted to a hyposmotic challenge (Fig. 2). Both oxonol derivatives used, (DISBAC2(3) and WW-781), inhibited bJ_{taur}. At a concentration of 10 μM, WW-781 and DISBAC2(3) inhibited bJ_{taur} by 50% and 43% respectively. $K_{0.5}$ values of 3 ± 1 μM were found for WW-781 (*not shown*) and 5 ± 1 μM for DISBAC2(3) (Fig. 3*A*).

Effect of Oxonol Derivatives and Ketoconazole on Cell Volume Regulation

In a previous study, we found that NPPB and DIDS blocked cell volume regulation (regulatory volume decrease, RVD) in isolated A6 cells submitted to a hyposmotic challenge (Ehrenfeld et al., 1994). Considering the inhibitory effects of the two oxonol derivatives and ketoconazole on taurine effluxes, we

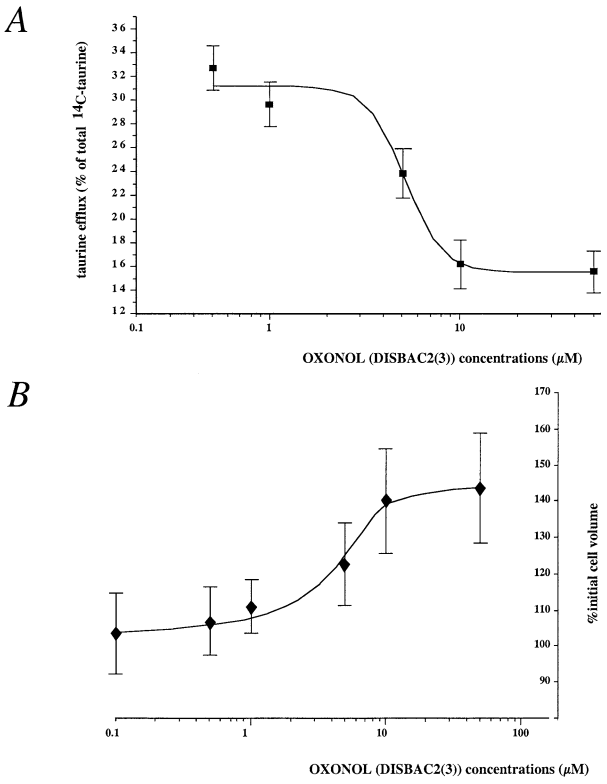

Fig. 3. Dose-response relationship of the inhibitory effect of DISBAC2(3) on taurine efflux (bJ_{taur}) and cell volume regulation in A6 cells. The dose-response relationships of the inhibitory effect of DISBAC2(3) on (bJ_{taur}) (*A*) and on cell volume regulation (*B*) are determined after switching the serosal bathing solution to a hyposmotic solution. The % of changes in cell volume were measured after 40 min of hyposmotic shock ($n = 4$) (*B*) while bJ_{taur} were measured over a 60-min period ($n = 6$) (*A*).

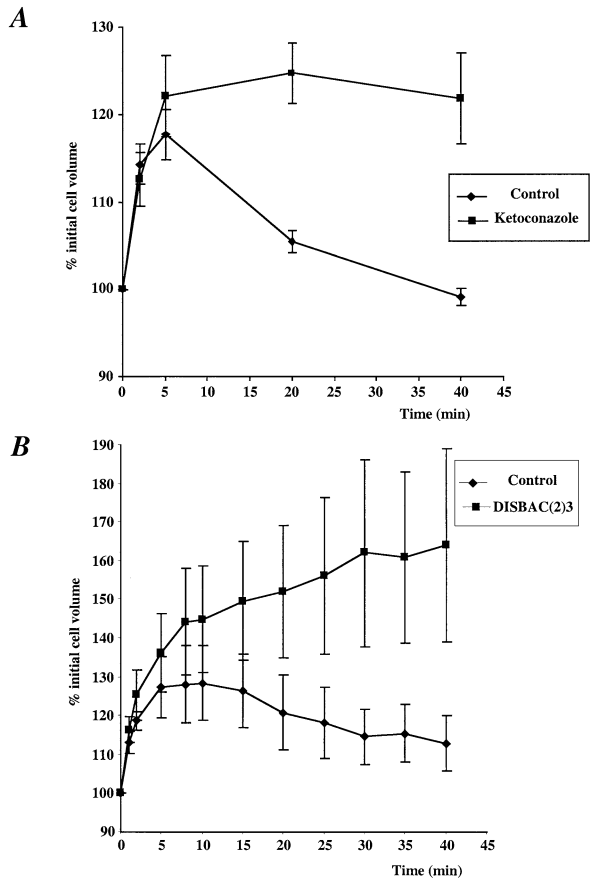

Fig. 4. Effects of ketoconazole and DISBAC2(3) on cell volume regulation in A6 cells. The effects of 100 μM ketoconazole (*A*), $n = 8$, and 100 μM DISBAC2(3) (*B*), $n = 4$, on cell volume regulation were measured as a function of time after the serosal bathing solution was changed to a hyposmotic solution.

investigated the effects of these agents on RVD. As shown in Fig. 4*A*, 100 μM ketoconazole increased the maximal cell swelling and completely blocked RVD. DISBAC2(3) at a concentration of 20 μM also inhibited RVD (Fig. 4*B*). A $K_{0.5}$ value of 5 ± 1 μM was estimated from the decrease in RVD observed over a range of DISBAC2(3) concentrations from 0.1 to 50 μM (Fig. 3*B*). Given the small changes in cell taurine concentration during RVD, the inhibitory effect of oxonol derivatives on the taurine transport pathway cannot account for the blockade of RDV. This is further supported by the fact that DISBAC2(3) or ketoconazole incompletely block the taurine efflux (Fig. 2), but completely block RVD. It is likely that the oxonol derivatives block RVD mainly through their effect on the chloride conductance.

Characterization of the Basolateral Taurine-Transport Pathway in Monolayers with Nystatin-permeabilized Apical Membranes

To further characterize the basolateral taurine efflux, we permeabilized the apical membranes of the A6 cell monolayers with the ionophore nystatin. This approach has already been successfully used with A6 cells (Rokaw et al., 1996; Fisher, Grillo & Sariban-Sohraby, 1996). We first verified that nystatin treatment (50 μM) effectively permeabilized the apical membranes by measuring the taurine efflux through these membranes. As already mentioned, the apical taurine permeability in untreated cells is very low (*see* Fig. 1). Less than 0.1 ± 0.1% ($n = 16$) of the initial cell taurine content is lost after 10 min. In nystatin-treated cells ($n = 4$), the loss of ^{14}C-taurine through the apical membrane increased to 73 ± 4% of the initial cell taurine content in the first 10 min and reached 92 ± 5% after 20 min. This experiment provides evidence that nystatin induced a considerable increase in the apical membrane permeability of A6 cells.

Next we examined the effect of isosmotic solutions of different ionic strength on the basolateral taurine transport pathway. The basolateral taurine efflux was assessed before and after 30 min of permeabilization with 50 μM nystatin, in either a

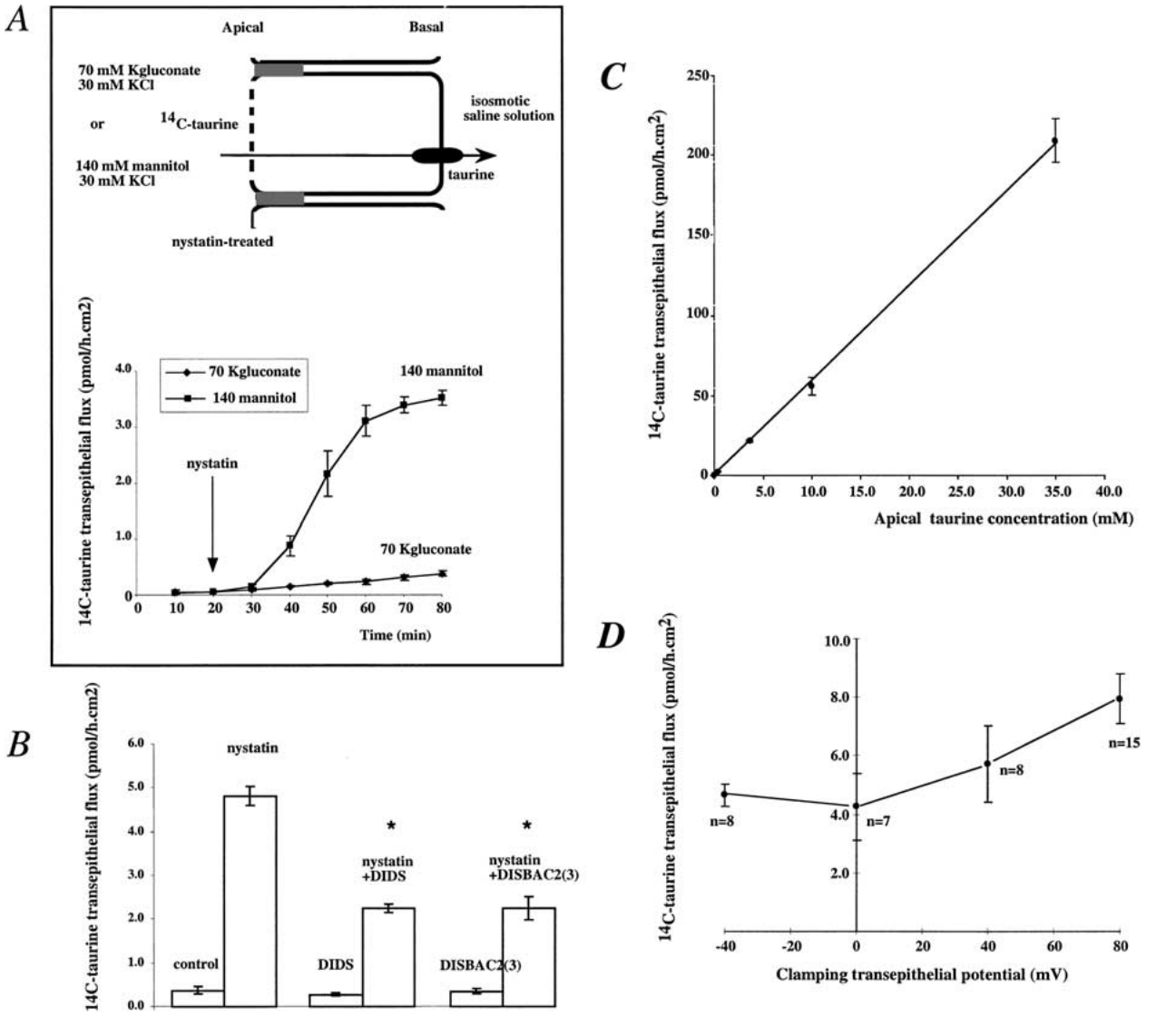

Fig. 5. Transepithelial taurine fluxes in isosmotic condition. (*A*) Nystatin (50 μM) was used to permeabilize the apical membranes of confluent monolayers as indicated by the arrow (20 min). The transepithelial taurine transport (m/sec) was stimulated when the apical medium was a 140 mM mannitol solution compared to a 70 mM Kgluconate solution (for detailed composition of the solutions, *see* Methods). The osmolarity of all solutions was maintained constant at 247 mOsm. (*B*) Effects of DIDS and DISBAC2(3) on the transepithelial taurine transport (m/sec) in the presence of the 140 mM mannitol solution (for complete composition, *see* Methods).* $p < 0.001$ (values compared to nystatin alone; $n = 4$). (*C*) Dependence of the taurine fluxes on the cellular taurine concentration. The transepithelial taurine transport (m/sec) was assessed at different taurine concentrations in the apical medium. Apical taurine concentrations varied from 3.5 μM to 35 mM in 140 mM mannitol solution and the A6 cell monolayers were permeabilized with 50 μM nystatin for 30 min ($n = 3$ for 3.5 and 35 μM; $n = 2$ for 350 μM, 35 mM and 10 mM, and $n = 5$ for 3.5 mM). (*D*) Dependence of the taurine fluxes on the membrane potential. Cells were incubated with 50 μM nystatin in the apical medium ("mannitol solution") for 30 min. The transepithelial taurine fluxes were measured at the indicated clamping voltages (referenced to the mucosal side). Significance: $p < 0.02$ between +80 mV and 0 mV, $p < 0.002$ between 80 mV and −40 mV; *NS* (non significant) between other paired data.

Kgluconate or a mannitol solution. As illustrated in Fig. 5*A*, in a 70 mM Kgluconate solution, the taurine fluxes (m/sec) were low in the absence of nystatin (0.06 ± 0.01 pmole/hr.cm^2, $n = 4$), increasing only slightly to 0.24 ± 0.04 pmole./hr.cm^2, $n = 4$ after 30 min of nystatin application. When a mannitol-containing solution was used in the absence of nystatin, the taurine fluxes (m/sec) were similar to those obtained with the 70 mM Kgluconate solution, remaining at low values (0.07 ± 0.01 pmole/hr.cm^2, $n = 4$). However, taurine fluxes (m/sec) were strongly stimulated after nystatin application, increasing to 3.11 ± 0.28 pmol/hr.cm^2, $n = 4$ after 30 min of ionophore application. Thus, the basolateral membrane taurine permeability was 13 times larger with a mannitol solution than with a Kgluconate solution.

There are two mechanisms, which are not mutually exclusive, that could underly the stimulation observed with the mannitol solution. First, the reduction of the intracellular ionic strength in the presence of the mannitol solution could trigger the opening of the taurine-transporting pathway, as reported in other cells (Cannon, Basarappa & Strange, 1998; Voets et al., 1999; Guizouarn & Motais, 1999). Second, the penetration of mannitol into the cells via nystatin pores could drive water into the cell and result in isosmotic cell swelling. The possibility of an isosmotic cell swelling was verified with cell-volume measurements using a Coulter counter. The experiments showed that in the presence of nystatin, the exposure to the mannitol-containing medium induced a large swelling of the cells (*data not shown*). Cell swelling was not observed in the absence of nystatin. In the presence of the Kgluconate solution, nystatin treatment induced only a moderate cell swelling. Therefore, the basolateral taurine transport pathway is stimulated by isosmotic cell swelling and the intracellular low ionic strength may potentiate this effect.

As reported in Fig. 5*B*, the ^{14}C-taurine flux elicited in nystatin-treated cells in the presence of the mannitol solution was inhibited by DIDS and DISBAC2(3) to a similar extent as the hyposmotically-induced taurine efflux. This suggests that the taurine efflux elicited in isosmotic conditions corresponds to the same transport pathway as in hyposmotic conditions. Next, we determined the basolateral taurine fluxes (m/sec) over a wide range of taurine concentrations, varying from 3.5 μM to 35 mM, in nystatin-treated cells exposed to the mannitol solution. As reported in Fig. 5*C*, the taurine fluxes (m/sec) did not appear to saturate with increasing solute concentration ($r = 0.99$). This behavior is consistent with permeation through ion channels.

Next, we examined the dependence of the basolateral taurine transport on the membrane potential. Apical nystatin application considerably reduces the apical membrane resistance. Thus, clamping the transepithelial voltage of the A6 cell monolayer results in clamping the most resistive barrier, i.e., the basolateral membrane. We determined the effect of basolateral membrane voltage changes on the basolateral taurine pathway. Taurine fluxes were assessed at different clamping voltages (ranging from −40 mV to +80 mV) after a 30-min nystatin-equilibration period (under open circuit conditions, mean transepithelial voltage and resistance were +48 ± 3 mV and 3411 ± 171 Ω.cm^2 before nystatin application, and 33 ± 2 mV and 1440 ± 114 Ω.cm^2, $n = 11$ after 20 min of nystatin application). The voltage-clamping periods directly followed the open-circuit periods and the order of the imposed voltages was changed randomly in the different experiments. As shown in Fig. 5*D*, the taurine fluxes were stimulated significantly by clamping the serosal side of the monolayer only for values above +40 mV.

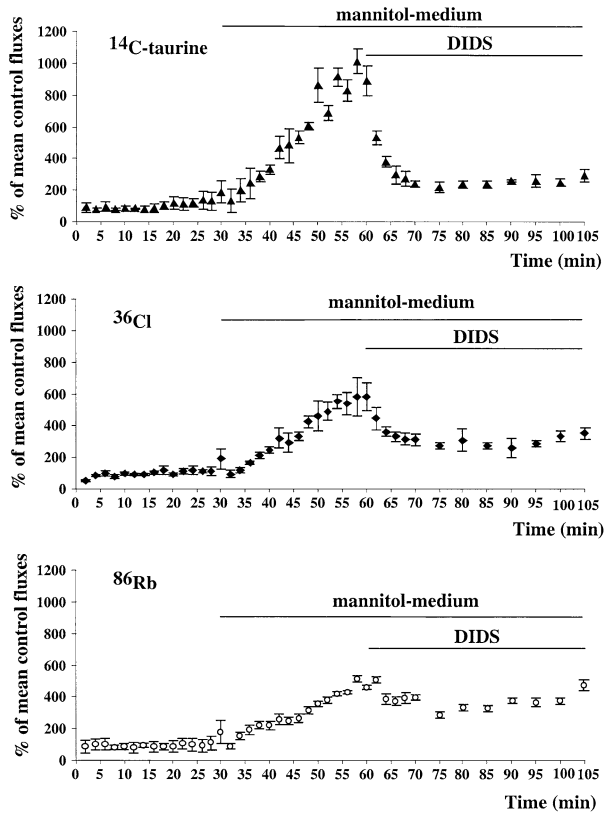

Fig. 6. Time courses of stimulation or inhibition of ^{14}C-taurine, ^{36}Cl and ^{86}Rb transepithelial fluxes. The cell monolayers were permeabilized with nystatin (50 μM). After 30 min, transports were stimulated by changing the apical medium (70 mM Kgluconate) to a 140 mM mannitol solution. DIDS (500 μM) was added to the serosal medium at 60 min. Changes in the transepithelial fluxes of ^{14}C-taurine, ^{36}Cl and ^{86}Rb are expressed relative to the mean value of the control period (0–30 min); $n = 4$. Experimental points every 2 min (or 5 min after 70 min) in open-circuit conditions.

Simulation and Inhibition Kinetics of ^{14}C-Taurine ^{36}Cl and ^{86}Rb Fluxes in Nystatin-permeabilized A6 Cell Monolayers

Whether the taurine and chloride transport pathways activated in RVD are identical or distinct is a controversial subject (*see* Discussion). In order to discriminate between these two possibilities, we measured simultaneously the chloride, taurine and potassium (m/sec) fluxes in apically permeabilized monolayers. We sequentially focused on the time courses of 1) transport stimulation and 2) transport inhibition after DIDS application. A typical experiment is shown in Fig. 6. Cells were preincubated with nystatin for 30 min prior to the change of the apical incubation medium from a Kgluconate- to a mannitol-containing solution. This resulted in immediate

increases of the transepithelial ^{14}C-taurine, ^{36}Cl and ^{86}Rb fluxes. The time courses of these stimulations were similar for the three solutes. DIDS application rapidly and simultaneously reduced the transepithelial fluxes of ^{14}C-taurine, ^{36}Cl and ^{86}Rb. However, the % of inhibition after 20 min of DIDS application was greater for the ^{14}C-taurine transport (71.3 ± 2.5%) than for ^{36}Cl (51.6 ± 2.6%) and ^{86}Rb (30.0 ± 4.7%) transport.

Since the experiments were carried out in open-circuit conditions, the inhibitory effect of DIDS on the ^{86}Rb transport could be indirect, i.e., through electrical coupling with chloride. We therefore compared the effect of DIDS on the ^{86}Rb transepithelial fluxes in open circuit and in A6 cell monolayers clamped at a potential of +40 mV. Under open-circuit conditions (mean transepithelial voltage 25.7 ± 1.7 mV), an inhibition of 36.1 ± 2.9% (n = 6) was found, whereas a 27.2 ± 5.7% (n = 6) reduction was observed under clamping conditions. Therefore, an inhibitory effect was still observed in clamping conditions.

Effect of DISBAC2(3) on the Hyposmotically-activated Chloride Conductance

We also determined the effect of DISBAC2(3) (10 μM) on the current activated by a hyposmotic challenge in A6 cells by patch clamp using the whole-cell configuration. Potassium conductances were abolished by replacing KCl by CsCl in the pipette solution and adding TEA in the extracellular bath. Sodium was replaced by NMDG in order to eliminate sodium currents. In all experiments, ATP(2 mM) was added to the pipette solution. Cell capacitance measured after reaching the whole-cell configuration was 17.5 ± 1.1 pF (n = 30). In 24 out of the 25 tested cells, a strong outwardly-rectifying current was observed in response to voltage pulses ranging from −80 to +100 mV. The reversal potential was found to be around −10 mV, a value close to the calculated equilibrium potential for chloride ions (E_{Cl} = 7 mV). External application of the hyposmotic solution (165 mOsm/1, obtained by omission of mannitol) resulted in a dramatic and reversible increase in current amplitude (375 ± 83 %, n = 8, P < 0.001, Fig. 7*A*). After the hypotonic-activated current reached a stable value, application of DISBAC2(3) induced a strong, rapid and reversible decrease in the chloride current (61.2 ± 10.6 %, n = 3; Fig. 7*A*). Neither the kinetic properties nor the rectification profile of the current were altered by DISBAC2(3) (Fig. 7*B*, *C*). Inhibition ratios remained constant over the entire voltage range used in the experiments (−80 to +100 mV), indicating that the blocking effect of DISBAC2(3) was not voltage-dependent (Fig. 7*C*).

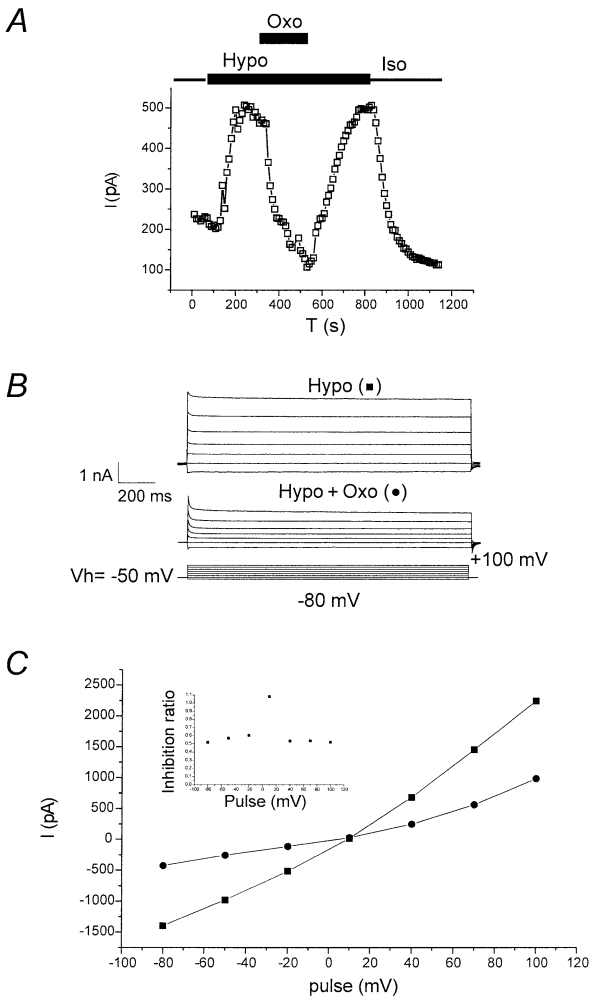

Fig. 7. Effects of DISBAC2(3) on the chloride current activated by a hypo-osmotic shock in A6 cells. (*A*) Time course of the change of the chloride current amplitude in response to a 75-mOsm hypotonic shock in the presence of DISBAC2(3) (10 μM) in the external solution. Perfusion times with the isosmotic and hyposmotic solution are represented by the thin (*Iso*) and the thick black bars (*Hypo*), respectively. DISBAC2(3)-containing hyposmotic solution was applied as indicated by the thick upper bar (*Oxo*). The holding potential *Vh* was −50 mV. (*B*) Chloride currents recorded from an A6 cell perfused with the hyposmotic solution in response to a series of voltage pulses (2 s each) ranging from −80 to +100 mV in 30 mV increments. Currents were obtained in the absence (square) or in the presence (circle) of 10 μM DISBAC2(3) (Oxo) in the external solution. (*C*) Corresponding *I*/*V* relationships obtained from the voltage protocol described above. The inset shows the current inhibition ratio calculated for each voltage pulse value.

Discussion

Taurine's Physiological Role(s) in Amphibian A6 Cells

Intracellular taurine concentration was found to increase when the cell monolayers were adapted to a hyperosmotic medium and to decrease when adapted to a hyposmotic medium. Under the three different

osmotic conditions tested, the intracellular taurine concentration was found to be larger than that of the extracellular medium (1 mM). As reported for other kidney cell lines (Jones, Miller & Chesney, 1990), this amino-acid accumulation is due to the functioning of a Na/Cl/taurine cotransporter (Ehrenfeld et al., unpublished data). The low taurine concentration in cells adapted to hyposmotic conditions (165 mOsm), suggests that taurine loss plays a minor role in cell volume regulation under this experimental condition. This is consistent with a previous study reporting a small concentration decrease (8 mM) of a pool of six different amino acids following a 30-min hypsomotic challenge (De Smet et al., 1995b; in that study, taurine was not measured). Indeed, it was shown that upon a hyposmotic challenge, RVD was mainly due to the loss of inorganic ions, i.e., K^+ and Cl^- (Ehrenfeld et al., 1994; De Smet et al., 1995a; Nilius et al., 1995). Conversely, under hyperosmotic conditions (327 mOsm), we found that the taurine concentration reached values as high as 7.3 mmole/l cells (a value close to 10 mM when expressed by cell water). These data strongly suggest that in A6 cells, as in many other cell types, taurine behaves like an organic osmolyte contributing to cell osmolarity changes in anisosmotic conditions.

What is the physiological function of taurine accumulation in cells? A role for taurine in RVD has been proposed in many cell types. Even though the amount of taurine released during RVD is small, together with other organic osmolytes, it could contribute significantly to RVD.

Several additional roles have been suggested for taurine in mammalian cells (for review *see* Huxtable, 1992). In MDCK cells, betaine and taurine have been found to exert a protective role against apoptosis induced by hypertonicity (Horio et al., 2001). The protective role of taurine is not restricted to renal cells and has been found in other cell types (Redmond, Wang & Bouchier-Hayes, 1996; Waters et al., 2001). This protective function of taurine could be important in amphibians, like toads, when living in a terrestrial environment. These animals can be exposed to dehydration and respond by increasing the osmolarity of their "milieu intérieur" (Garcia-Romeu, Masoni & Isaia, 1981). It is therefore possible that in A6 cells, taurine plays such a protective function under hyperosmotic conditions.

Recently, another physiological role for taurine has been suggested by Brochiero et al., (2002). These authors have demonstrated that in rabbit proximal tubule, taurine blocks a member of the K_{ATP} channel family that functions at the basolateral membrane. Thus, the loss of intracellular taurine during RVD could release the inhibition of basolateral K_{ATP} channels, which could contribute to the recovery of cell volume. The presence of K_{ATP} channels has been suggested in A6 cells (Ehrenfeld et al., 1994) and, as in proximal tubule cells, these channels could be negatively controlled by taurine. Furthermore, in Na^+ transporting epithelia, the apical Na^+ conductance and basolateral K^+ conductance are tightly coupled by "cross-talk" mechanisms (*see* Harvey, 1995: Schultz, Dubinsky & Lapointe, 1998). Thus, the increase in cell taurine during hyperosmotic adaptation, which leads to the inhibition of K_{ATP} channels, could contribute indirectly to the reduction of transepithelial Na^+ transport in amphibian epithelia adapted to high-Na^+-containing media (Katz, 1975; Ehrenfeld, Lacoste & Harvey, 1989).

Permeability of Apical and Basolateral Membrane to Taurine

The A6 epithelium presents a polarized distribution of transporters and channels involved in transepithelial sodium and chloride transport, as well as in cell volume regulation. Our study demonstrates that the basolateral membranes of A6 cells maintained in isosmotic medium have a considerably higher permeability to taurine than the apical membranes. This difference in membrane permeability is even more pronounced when the basolateral membranes are exposed to a hyposmotic solution. Therefore, the taurine transport pathways are also distributed in a polarized fashion.

In our study, the loss of organic osmolytes (such as taurine) through a basolateral pathway in response to hyposmotic challenge appears to be complementary to the loss of the inorganic ions reported previously (Ehrenfeld et al., 1994; De Smet et al., 1995a; Nilius et al., 1995), which account for the majority of osmolytes that are lost. The stimulation of the taurine transport pathway by very small osmotic changes (4%) in the external medium suggests a very acute sensitivity of this regulatory mechanism.

Characteristics of the Basolateral Taurine Pathway

Permeabilization of the apical membrane by nystatin allowed us to define several characteristics of the basolateral taurine pathway. The basolateral taurine transport was found to be stimulated in isosmotic conditions by using an apical medium of low ionic strength and containing mannitol. The stimulation of taurine transport by low ionic strength is consistent with previous studies reported on different cell types (Cannon et al., 1998, Voets et al., 1999, Guizouarn & Motais, 1999). However, we also observed an isosmotic cell swelling in the presence of this osmolyte and it is therefore not clear whether the low ionic strength is the unique cause of the stimulation of the taurine efflux or if another event related to the swelling is involved. Electrophysiological studies have revealed that taurine transport can generate a current

(in solutions with alkaline pH in which the amino acid is negatively charged) and is mediated by a swelling-activated anion channel (Banderali & Roy, 1992, Kirk et al., 1992). However, at a physiological cell pH (7.2 to 7.4) the transport of taurine in its zwitterionic form is more probable. This was clearly demonstrated in fish red blood cells, in which the magnitude of the taurine concentration changes implied the transfer in the zwitterionic form (Guizouarn et al., 2000). We found that in nystatin-permeabilized monolayers, the transepithelial taurine fluxes were stimulated for a clamping potential larger than +40 mV. A similar voltage dependence of the taurine transport was also reported in HeLa cells (Stutzin et al., 1999) and in Ehrlich mouse ascites tumor cells (Lambert & Hoffmann, 1994). The swelling-activated taurine efflux in these cells was voltage dependent with higher efflux rate constants at hyperpolarizing potentials. Since the driving force for taurine (in its zwitterionic form) is not changed at hyperpolarizing voltages, it is likely that the slight stimulation of the taurine efflux at hyperpolarizing voltages observed in A6 cells is due to the voltage dependence of the transporter (or channel) itself.

Another particularity of the A6 taurine pathway is its linear dependence on taurine concentration (measured over a range of concentrations from 3.5 μM to 35 mM). This failure to saturate the transport pathway with increasing concentrations of substrate had already been reported in cultured renal papillary epithelial cells (Siebens & Spring, 1989), fish erythrocytes (Kirk et al., 1992) and brain glial cells (Strange et al., 1993) and suggests that the taurine transport pathway consists of a channel or a pore rather than a conventional transporter.

Drug Sensitivity of the A6 Taurine Pathway

The drug sensitivity of the taurine pathway in A6 cells presents some similarities to, but also marked differences with other cell types. bJ_{taur} was not blocked by 1,9-dideoxyforskolin, tamoxifen and verapamil. Therefore, the P-glycoprotein blockers tested were ineffective in taurine transport inhibition in A6 cells, suggesting that P-glycoprotein is not involved in taurine transport in A6 cells.

Unexpectedly, verapamil stimulated the swelling-activated ^{14}C-taurine effluxes. Since this stimulation was not sensitive to DIDS, we considered this pathway as nonspecific and different from the hyposmotically-stimulated taurine pathway. The existence of an additional, DIDS-insensitive, taurine pathway could explain the incomplete inhibition of taurine efflux found with some drugs (*see* Fig. 2) that totally blocked RVD (ketoconazole for instance).

A similar stimulation of taurine efflux by verapamil has also been reported in human tracheal 9HTEo cells by Galietta, Romeo & Zegarra-Moran, (1996). These authors excluded indirect effects, such as an inhibition of voltage-dependent Ca^{2+} channels or K^+ channels. In A6 cells, the origin of the verapamil stimulation remains unknown. One possibility is the inhibition of potassium channels by verapamil, as reported in other epithelia (Galietta et al., 1991; Hillyard & Van Driessche, 1992). Consistent with this hypothesis, verapamil was found to impair RVD in A6 cells (De Smet et al., 1998). TEA, another K^+ channel blocker, was also found to impair RVD (Ehrenfeld et al., 1994) and to increase the taurine efflux (our unpublished data). The block of K^+ channels by verapamil could increase the taurine efflux through the depolarization of the basolateral membranes. However, we and others (*see* above) found that the taurine efflux was slightly sensitive to membrane potential, being increased by hyperpolarization, therefore rendering this mechanism rather unlikely. Another explanation would be the continuous stimulation of the cell volume sensor mechanism when RVD is impaired by the K^+ channel block. Considering the high sensitivity of the taurine pathway to this sensor, an increased passage of taurine (in its zwitterionic form) would be expected, but not that of chloride, which would require the simultaneous passage of potassium. The small contribution of taurine to RVD is compatible with the impairment of cell volume recovery in the presence of verapamil or TEA.

The sensitivity of the taurine transport pathway to DIDS and NPPB has been found in several cell types including human lung cancer cell line S1 (Kirk & Kirk, 1993), rat C6 glioma cells (Jackson & Strange, 1993), cultured astrocytes (Sanchez-Olea et al., 1993), HeLa cells (Stutzin et al., 1999) or endothelial cells (Manolopoulos et al., 1997). A voltage dependence of DIDS inhibition of a volume-sensitive chloride current has also been reported previously, the effect of DIDS being particularly weak at hyperpolarizing potentials (Diaz et al., 1993; Shuba et al., 2000). The basolateral membranes of A6 cells are expected to be hyperpolarized in response to hyposmotic conditions. Therefore, if the taurine and chloride effluxes are mediated through a common transport pathway, it would be expected that the inhibitory effect of DIDS on the swelling-activated taurine transport is weak. In our study, this was not the case. One possible explanation may be that the high concentration of DIDS that we used (500 μM) may have compensated for the DIDS-voltage dependence. Another possibility would be the existence of separate pathways for taurine- and Cl^--osmosensitive fluxes. This has previously been suggested (for reviews *see* Kirk, 1997; Hoffmann, 2000). In HeLa cells, Stutzin et al., (1999) found distinct sensitivities to DIDS and different time courses of the inhibition of the Cl^- conductance and the taurine permeability. These results led the authors to suggest distinct pathways for Cl^- and taurine. Our

experiments were not as conclusive, since the taurine transport was found to be more sensitive to DIDS (71% of inhibition) than the ^{36}Cl transport (52% of inhibition), but no difference in the time courses of inhibition was found for these two solutes. However, the difference in DIDS sensitivity found for the taurine and the Cl^- transports may be associated with the presence of four different Cl^- channels in A6 cells stimulated by hyposmotic conditions (Banderali et al., 1996). Indeed, these channels could present different sensitivities to DIDS and different permeabilities to taurine. Therefore, the difference in sensitivity to DIDS would be consistent with taurine permeation through one or more, but not all, of the four Cl^- channels activated in hyposmotic conditions. Oxonol derivatives were reported to inhibit Cl^- channels and Cl/HCO_3 exchangers (Arreola, Hallows & Knauf, 1995; Alper et al., 1998). In the present study, an inhibitory effect of two oxonol derivatives, WW791 and DISBAC2(3), was also found on Cl^- currents stimulated by hyposmotic challenge with A6 cells patched in the whole-cell configuration. The blocking effect of oxonol was not voltage-dependent (*see* Fig. 7*C*), in contrast to DIDS (*see* above). We also demonstrated the inhibition of bJ_{taur} by WW791 and DISBAC2(3) with approximate $K_{0.5}$ values of 3 $\pm$ 1 and 5 $\pm$ 1 μM, respectively. These relatively low $K_{0.5}$ values classify the oxonol derivatives among the most selective agents compared to the previously reported taurine inhibitors. We also report in this study the inhibition of cell volume regulation by the two oxonol derivatives, (WW-791 and DISBAC2(3)) with $K_{0.5}$ similar to those observed with bJ_{taur}. The block of RVD can mainly be attributed to the inhibition of the Cl^- loss since intracellular taurine changes are small. Nevertheless, the inhibition by oxonol derivatives of the taurine as well as the Cl^- transport argues in favor of a common transporting pathway for these solutes.

In conclusion, we describe the pharmacological characteristics of the swelling-activated taurine pathway in A6 cells, which is located on the basolateral membranes of this highly polarized epithelium. We report the inhibition of the swelling-activated taurine and Cl^- transports by two oxonol derivatives. These agents represent useful inhibitors for future studies aiming to identify the molecular nature of the transporting protein(s). Our experiments do not allow the discrimination between the chloride- and the taurine-transport pathways, suggesting that a common pathway mediates anion and organic osmolyte transport. We do not exclude that additional, more selective chloride channels participate in cell volume regulation in A6 cells.

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