Amino Acids (1997) 12: 57–75

Amino
Acids

Taurine transport systems in the ciliate protozoan *Tetrahymena pyriformis*

B. Kramhøft* and **I. H. Lambert**

Biochemical Department, August Krogh Institute, University of Copenhagen, Copenhagen, Denmark

Accepted July 9, 1996

Summary. *Tetrahymena pyriformis* cultivated in the presence of 1 mM taurine prior to transfer of the cells to non-nutrient medium express an enhanced capacity for concentrative taurine uptake and for taurine diffusion compared to cells grown without added taurine. The unidirectional taurine influx in taurine-grown cells comprises a saturable component with K_m = 257 μM, V_{max} = 21 nmoles · g dry wt^{-1} · min^{-1}, and a diffusion component with a diffusion constant of 0.20 ml · g dry wt^{-1} · min^{-1}. At extracellular taurine concentrations <30 μM, 20% of the influx is via the saturable system and 80% is via the diffusion system. 19% of the influx in Na^+-*dependent*, Cl^--*independent*, and not inhibitable with structural analogues to taurine, suggesting that the transport system responsible for the saturable component in *Tetrahymena* is different from the Na^+- and Cl^--dependent taurine translocating system (the β-system) described in vertebrate cells. The unidirectional taurine influx is reduced by 80% by 1 mM DIDS (inhibitor of anion exchange and anion channels) and by 1 mM MK196 (indachrinone, inhibitor of anion channels) indicating that taurine diffusion in *Tetrahymena* is via a channel, which is permanently active and which resembles the swelling-induced "taurine channel" seen in mammalian cells. Taurine influx is stimulated by the forskolin analogue 1,9-dideoxyforskolin and by arachidonic acid, and this stimulation is in both cases sensitive to DIDS and MK196.

Keywords: Amino acids – Taurine channels – Arachidonic acid – DIDS – 1,9-Dideoxyforskolin – Indachrinone

Abbreviations: DDF, dideoxyforskolin; DIDS, 4,4′-diisothiocyano-2,2′-stilbene disulfonic acid; GABA, gamma amino butyric acid; HEPES, N(2-hydroxyethyl)piperazine-N′-(2-ethane sulfonic acid); MK196, indachrinone;

* Present address: Carlsberg Laboratory, Chemical Department GL. Carlsbergvej 10, DK-2500 Jalby.

MOPS, 3-(N-morpholino)propane sulfonic acid; NMDG, n-methyl-d-glucamonium; OPA, ortho-phtalaldehyde; PCA, perchloric acid; TES, N-tris(hydroxy methyl)-methyl-2-amino ethane sulfonic acid; TRIS, tris(hydroxy methyl)amino methane.

Introduction

Taurine (2-amino ethane sulphonic acid) is a constituent of the cytosolic amino acid pool in most cells and has been assigned several physiological functions such as intracellular osmolyte, membrane stabilizer, modulator of Ca^{2+} effects, and modulator of membrane excitability (see Huxtable, 1992). Active taurine transport in vertebrate cells is well described (see Huxtable, 1992) and is as for example in the Ehrlich ascites tumor cell, typically mediated by a high affinity, Na^+- and Cl^--dependent transport system (the β-system), which is inhibited by structural analogues to taurine such as β-alanine and hypotaurine (Lambert, 1984, 1985). In addition, taurine channels, mediating taurine diffusion, are activated in many cells following hyposmotic cell swelling (see Lambert and Hoffmann, 1994; Strange and Jackson, 1995).

In contrast to the general abundance of taurine in the animal kingdom, the taurine content of protozoa is low (Huxtable, 1992). This is also true for the ciliate *Tetrahymena*, where it was previously found that the amino acid pool contained only a small amount of taurine (Scherbaum et al., 1959). Taurine has, however, been identified in *Tetrahymena* grown in a broth medium as a component of a group of membrane lipids termed taurolipids. These lipids comprise various conjugates of stearic acid (octodecanoic acid) with taurine, and their biosynthesis has previously been described (Kaya et al., 1985; Kaya and Sano, 1991). A similar group of taurolipids has recently been identified in the flagellate *Euglena* (Saidha, 1993). Although taurolipids constitute about 1% of the total lipid fraction of *Tetrahymena* (Kaya et al., 1985), their physiological function has yet to be established. As suggested by Saidha (1993) for *Euglena*, they may serve as membrane protectants.

Since the biosynthetic pathway of the taurolipids in *Tetrahymena* was identified using exogenously added ^{14}C-taurine (Kaya and Sano, 1991), taurine must have been taken up by the cells prior to incorporation in the lipid fraction. In agreement with this, amino acid transporting systems similar to systems found in higher organisms were previously demonstrated in *Tetrahymena* (Hoffmann and Rasmussen, 1972). Amino acid transport in protozoa including *Tetrahymena* has to the knowledge of the present authors last been reviewed by Aomine (1981).

Tetrahymena may in many respects serve as a model for the physiology of higher organisms (Wheatley et al., 1994), and since *Tetrahymena* has a mammalian-like requirement for amino acids (see Hill, 1972), it was of relevance to study taurine uptake by *Tetrahymena* and the anticipated transport systems. The present study confirms that taurine is taken up by *Tetrahymena*

from the extracellular medium and the paper characterizes the involved transport systems with respect to ion-dependence, specificity, and pharmacology. Preliminary results have been published as an abstract at the 4th International Congress on Amino Acids in Vienna, August 1995 (Kramhøft and Lambert, 1995).

Materials and methods

Culture conditions and media

Tetrahymena pyriformis, strain GL, was maintained by biweekly subcultivation at room temperature in test tubes containing 4 ml PPY growth medium without added glucose. The composition of PPY medium was as follows: 0.75% proteose peptone, 0.75% yeast extract, 100 μM ferric citrate, 50 μM $CaCl_2$, and 1 mM $MgCl_2$ (Hellung-Larsen, 1988). Experimental cultures were grown at 28°C in PPY medium with 1% glucose for 24–26 hrs, without added taurine or with 1 mM taurine, in a shaking water bath in 250 ml Erlenmeyer flasks containing 80 ml cell culture. Glucose and taurine were added from separate concentrated sterile stock solutions. At the time of harvest the cultures had reached early stationary growth phase ($0.5–0.8 \cdot 10^6$ cells $\cdot$ ml^{-1}). They were then briefly chilled and subsequently transferred to an inorganic salt medium (IMP) by three centrifugations (700 g, 1 min) followed by resuspension to a final cell density corresponding to 30–60 mg cell wet weight $\cdot$ ml^{-1} (about $1–2 \cdot 10^6$ cells $\cdot$ ml^{-1}). IMP medium was modified from Hoffmann and Kramhøft (1969), and had the following composition (in mM): NaCl: 30; KH_2PO_4: 1; K_2HPO_4: 4; $MgCl_2$: 5; $CaCl_2$: 1; MOPS: 3.3; TES: 3.3, and HEPES: 3.3. In Na^+-free medium Na^+ was replaced by the equivalent concentration of Tris or NMDG-Cl, and in Cl^--free medium, Cl^- was replaced by the equivalent concentration of the respective gluconates. The pH of the media was 7.2. The cells were allowed to recover from the centrifugations by standing in a thin layer (10–15 ml cell suspension in a 100 ml Erlenmeyer flask) at room temperature for $^1/_2$ hr. Then the pH of the culture was readjusted to pH 7.2 and the experiment subsequently initiated.

Taurine analysis

A cell pellet containing 70–90 mg cells (wet weight) was homogenized in 0.5 ml 4% sulfosalicylic acid, placed on ice for 1 hr and then centrifuged for 10 min (2,000 g, 4°C) in order to remove proteins. The taurine content in the supernatant was estimated by a standard OPA derivatisation procedure followed by separation on a LKB HPLC system using a Sephasil column (C18, 5 μm, 4 * 250). Elution was performed with acetonitrile in a 12.5 mM phosphate buffer (pH 7.2), using a flow rate of 1.2 ml $\cdot$ min^{-1} and by increasing the acetonitrile concentration from 0% to 23.5% within 25 min and from 23.5% to 50% within the following 3 min. The taurine concentration in the supernatant was estimated from the UV absorption at 330 nm, using norvaline and taurine as internal and ordinary standards, respectively.

Taurine influx

At time zero influx was initiated by addition of ^{3}H-labelled taurine (3.7 kBq $\cdot$ ml^{-1}). The final taurine concentration is indicated in the figure legends. Samples (1 ml) of the cell suspension were then separated from the medium at the desired time points by differential centrifugation (20,000 g, 1 min) through a mixture of dioctylsebacinate (14.73 w/w%) in dibutylphtalate (Ballentine and Burford, 1960) in preweighed Eppendorf vials. Samples of the supernatants were saved for the determination of radioactive amino acid in the medium. The supernatant and the separation layer were then carefully removed, the

pellet lysed with 0.05% Triton X-100 and subsequently deproteinized with PCA (final concentration 7%). The deproteinized samples were centrifuged (20,000 g, 10 min) and the supernatant was saved for the determination of the content of ^{3}H-labelled taurine in the acid soluble cellular pool. The PCA precipitate was dried for 48 hrs at 80–90°C and weighed. In separate experiments it was found that PCA did not contribute significantly to the cell dry weight. The results were corrected for trapped extracellular medium (11% of the cellular wet weight) determined using ^{3}H-inulin as extracellular marker (Kramhøft and Jessen, 1992), and calculated as nmoles of taurine taken up per g cellular dry weight. Radioactivity was assessed by liquid scintillation counting.

Taurine efflux

Cells were grown as described above in the presence of 1 mM non-radioactive taurine plus ^{3}H-taurine (10 kBq·ml^{-1}). The cultures were then transferred to IMP by centrifugal washings as described above. Taurine efflux from *Tetrahymena* is relatively slow (see Fig. 8) and was monitored from the ^{3}H-radioactivity retained in the acid soluble cellular pool as a function of time after initiation of the experimental treatment.

Measurement of cellular ion concentrations

Cells were separated from the suspension medium as described above. The concentrations of Na^+ and K^+ in the medium and in the cells were assessed by atomic absorption flame photometry (Perkin Elmer model 2380). Cl^- was measured by coulometric titration (CMT 10 chloride titrator, Radiometer, Denmark). Cellular ion concentrations are given as mmoles·l cell water^{-1} (mM) after correction for extracellular trapped medium as described above.

Chemicals

Yeast extract and proteose peptone were from Difco (Detroit, Mi, USA). MK196 was a gift from Merck Research Laboratories (Rahway, NJ, USA), which is gratefully acknowledged. All other chemicals were from Sigma (St. Louis, Mo, USA). DDF, arachidonic acid and MK196 were dissolved in ethanol (at 10, 50 and 200 mM, respectively). The MK196 stock solution was prepared just prior to the experiment and discarded after use. The arachidonic acid stock solution was kept at −20°C under N_2 in order to avoid oxidation. DIDS was dissolved in water (10 mM) and kept frozen in small aliquots. Excess thawed DIDS stock solution was discarded. ^{3}H-inulin was from Amersham (Little Chalfont, England). ^{3}H-taurine was from Amersham or NEN (Du Pont de Nemours, Dreiech, Germany).

Statistical evaluation

Results are given either as one representative experiment out of at least 3 separate experiments or as the mean ± S.E.M. with the number of experiments in parenthesis. Error bars on the figures represent S.E.M. values. Unless otherwise indicated an observation is taken as significant when $P < 0.01$ in a Student's *t*-test.

Results

Identification of taurine in the cellular pool by HPLC

The taurine content of the acid soluble cellular pool in *Tetrahymena* grown under standard conditions (see Methods) is extremely low. Subjection of a cell extract to HPLC reveals that the cellular taurine concentration is below

0.05 mM. However, when *Tetrahymena* is cultivated in the presence of 1 mM taurine for 24 hrs the cytosolic acid soluble pool eventually contains a significant amount of taurine. The cellular taurine concentration is in this case estimated at 0.9 ± 0.1 mM (n = 3). Furthermore, the taurine gradient calculated from the distribution of ^{3}H-taurine between the cellular compartment and the extracellular medium ($[^3\text{H-Tau}]_i \cdot [^3\text{H-Tau}]_o^{-1}$) is 0.96 ± 0.08 (n = 5). Thus, the intracellular taurine concentration equals the extracellular taurine concentration after growth in the presence of 1 mM taurine. Figure 1 demonstrates a HPLC chromatogram of an extract of the cellular pool of cells, which were grown for 24 hrs in the presence of ^{3}H-taurine. It is seen that the chromatogram contains 2 peaks, which represents taurine (time 21 min) and methionine (time 28 min), respectively. The ^{3}H-labelled methionine, which occurred as an impurity of the ^{3}H-labelled taurine standard, thus seems to be taken up by *Tetrahymena*.

Transport systems for taurine

Taurine uptake was subsequently studied in *Tetrahymena* cultivated for 24 hrs in the absence or in the presence of taurine (1 mM) before transfer to taurine-free inorganic medium (IMP). Figure 2 depicts the cellular taurine content as a function of time after addition of ^{3}H-taurine. It is seen that taurine is taken up by cells grown in the absence of taurine (Fig. 2, open circles) as well as by cells grown for 24 hrs in the presence of taurine (Fig. 2, closed circles). In both cases the taurine uptake ceases at about 20 min after addition of the ^{3}H-

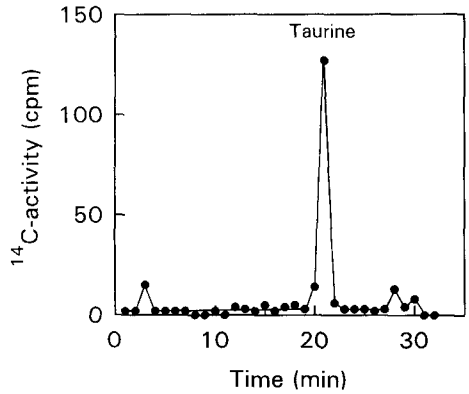

Fig. 1. HPLC profile of ^{3}H-activity from *Tetrahymena* grown for 24 hr in the presence of 1 mM taurine. *Tetrahymena* grown for 24 hr in PPY medium containing ^{3}H-labelled taurine (3.7 kBq·ml^{-1}) and 1 mM non-radioactive taurine, were prepared for taurine analysis as described in Methods. Elution was performed in a 12.5 mM phosphate buffer (pH 7.2) on a Sephasil column (C18, 5 μm) using a flow rate of 1.2 ml·min^{-1}, and by increasing the acetonitrile concentration in the mobile phase from 0% to 23.5% in the time range from 2 min to 27 min and from 23.5% to 50% in the time range from 27 min to 30 min. The elulate from the column was collected in 1.2 ml fractions on a LKB fraction collector and the ^{3}H-activity in each fraction was subsequently assessed on a liquid scintillation counter. The figure is representative of 4 sets of experiments

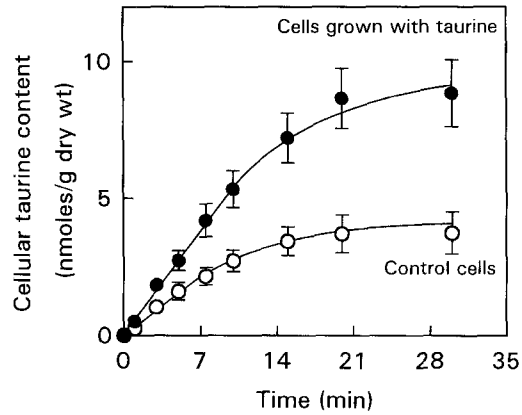

Fig. 2. Uptake of taurine as a function of time in *Tetrahymena* cultivated in the absence and in the presence of added taurine (1 mM), respectively. Cells were transferred to inorganic medium (IMP) as described in Materials and methods, ^{3}H-taurine (3.7 kBq·ml^{-1}) was added at time zero and samples were subsequently removed for the determination of radioactivity in medium and cellular acid soluble pool. The final taurine concentration is 1.1 μM in cultures grown without added taurine and about 1.3 μM in cultures cultivated in the presence of 1 mM taurine over night. The concentration difference arises from residual taurine from the taurine containing growth medium. The dilution of the original growth medium was assessed in separate experiments and is 5,000 fold. From experiments like the one shown in Fig. 3 it was calculated that the taurine concentration dependence of the initial taurine influx is 0.25 nmoles·g dry wt^{-1}·min^{-1} per μM. Hence, the influx values for taurine influx were corrected for the contribution of the 0.2 μM concentration difference before comparison, i.e. 0.05 nmoles·g dry wt^{-1}·min^{-1}. The figure shows the result of 11 parallel experiments

labelled taurine. However, the initial taurine influx, i.e. the influx measured within the first 7–10 min after addition of the isotope, is significantly higher in cells grown with added taurine, i.e. 0.60 ± 0.06 nmoles·g dry wt^{-1}·min^{-1} compared to 0.32 ± 0.04 nmoles·g dry wt^{-1}·min^{-1} (n = 11) in the controls grown without added taurine (see also legend to Fig. 2). Furthermore, the resulting gradient, calculated from the distribution of ^{3}H-taurine 30 min after addition of the isotope, is significantly larger in cells preexposed to taurine, i.e. 1.7 ± 0.2, compared to 0.8 ± 0.1 in the controls (n = 11). Thus, the gradient calculated in cells cultivated in the presence of taurine is significantly above 1, suggesting that active taurine transport occurs in *Tetrahymena* preexposed to taurine.

In order to investigate whether the taurine uptake expressed after cultivation in the presence of taurine was saturable, we determined the initial taurine influx as a function of the extracellular taurine concentration. A typical concentration dependence curve is shown in Fig. 3 (closed circles). Assuming that taurine uptake in *Tetrahymena* is via a non-saturable diffusion process plus a saturable component that conforms to Michaelis-Menten kinetics, and using a nonlinear regression analysis of the raw data, we estimated the diffusion constant, D, at 0.20 ± 0.02 ml·g dry wt^{-1}·min^{-1}. For the saturable system the apparent Michaelis-Menten constant, K_m, was estimated at 257 ± 90 μM, and the uptake capacity, V_{max}, at 21 ± 4 nmoles·g dry wt^{-1}·min^{-1} (n = 4). Thus, the

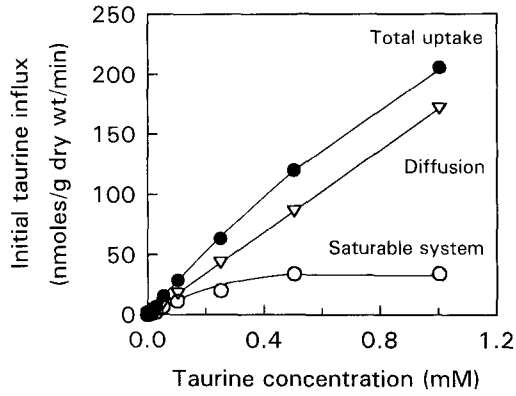

Fig. 3. Initial taurine influx as a function of the extracellular taurine concentration. Cells were grown overnight in the presence of 1 mM taurine. ^{3}H-taurine (3.7 kBq·ml^{-1}) was added in combination with non-radioactive amino acid at the concentrations indicated and duplicate samples were removed at time 7–8 min after addition of the isotope. The influx was calculated assuming linearity of the taurine uptake within this period. The "Total uptake" was treated as the sum of a single saturable component and a diffusion component. The influx (V) via the saturable system is described as $V = V_{max} \cdot [tau]_o \cdot (K_m + [tau]_o)^{-1}$, where V_{max} is the maximal influx, $[tau]_o$ the extracellular taurine concentration and K_m the taurine concentration required for half maximum influx. The diffusion influx is described as $V = D \cdot [tau]_o$, where D is the diffusion constant. The figure is representative of 4 individual experiments

saturable system in *Tetrahymena* has a low affinity for taurine as well as a low transport capacity. It is calculated from these experiments that at low extracellular taurine concentrations (below 30 μM) the influx via the saturable system amounts to 20 ± 7% (n = 4) of the total taurine influx.

In mammalian cells saturable taurine influx is mediated by the β-amino acid transporting system designated the β-system, which is Na^+- and Cl^--dependent, and inhibitable with structural analogues to taurine (e.g. β-alanine and hypotaurine; see Huxtable, 1992). In the case of Ehrlich cells it is the Na^+-gradient which provides the energy for active taurine influx and no taurine is taken up in the absence of extracellular Na^+ (Lambert, 1984). The ion composition of *Tetrahymena* cells suspended in the present salt medium is given in Table 1. *Tetrahymena* resembles mammalian cells by having a high cellular K^+ concentration, a low cellular Na^+ concentration and thus an inwardly directed Na^+ gradient. The membrane potential of *Tetrahymena* cells was previously estimated at about −25 mV (Connolly and Kerkut, 1983). Assuming that the apparently active taurine influx in *Tetrahymena* (Fig. 2) is via the saturable system (Fig. 3), this uptake could conceivably be via a system similar to the β-system and driven by the Na^+ gradient. Taurine uptake in *Tetrahymena* at low extracellular taurine concentrations should, therefore, be inhibited by about 20% following removal of Na^+ from the medium and reduced to a similar extent following removal of extracellular Cl^- or addition of structural analogues such as β-alanine, hypotaurine or GABA. Table 2 summarizes the results of experiments in which Na^+ or Cl^- were substituted for Tris or

Table 1. Na^+, K^+ and Cl^- concentrations in *Tetrahymena* and in IMP

	Na^+	K^+	Cl^-
Cellular concentrations (mmoles·l cell water^{-1})	6 ± 0.6	49 ± 0.6	6 ± 0.2
Extracellular concentrations (mmoles·l medium^{-1})	32 ± 0.2	14 ± 1	46 ± 1

Cultures were harvested and suspended in IMP as described in Materials and methods. Duplicate samples were taken 30 min after transfer to IMP for the measurement of extracellular and cellular concentrations of Na^+, K^+, and Cl^-. The values are averages from 4 separate experiments.

Table 2. Effect of substitution of extracellular Na^+ or Cl^- and the effect of hypotaurine, β-alanine and GABA on taurine influx in *Tetrahymena* grown in the presence of 1 mM taurine

	^{3}H-taurine influx (relative values)
Control	1
Na^+-free	0.81 ± 0.07* (6)
Cl^--free	1.10 ± 0.12 (5)
Hypotaurine	0.95 ± 0.05 (3)
β-alanine	0.99 ± 0.05 (3)
GABA	0.99 ± 0.05 (3)

Tetrahymena cultures were grown for 24–26 hrs in PPY medium containing 1 mM taurine. The cells were then transferred to IMP, Na^+-free IMP or Cl^--free IMP as required. Taurine influx was initiated by addition of ^{3}H-taurine (3.7 kBq·ml^{-1}, 1.3 μM) and samples were removed at the desired time points. In the ion substitution experiments the initial influx was calculated as nmoles·g dry wt^{-1}·min^{-1} by linear regression of measurements obtained within the first 7–10 min after addition of the isotope. In the experiments with the effect of structural analogues, hypotaurine, β-alanine or GABA were added just prior to ^{3}H-taurine at a final concentration of 1 mM. Duplicate samples of the cultures were harvested at time 7.5 min after addition of the isotope. The initial influx was calculated as nmoles·g dry wt^{-1}·min^{-1}. Controls and experimental cultures were always treated in parallel and due to day to day variations in the absolute flux values, the results are given relative to the control flux in parallel cultures. The numbers in parenthesis denotes the number of experiments in each case. * The value is significantly different from the control.

gluconate, respectively. Removal of Na^+ does, indeed, reduce the initial taurine influx significantly and by 19%. In one additional experiment Na^+ was substituted for $NMDG^+$ instead of Tris with almost the same result (15% inhibition). Removal of Cl^- has no significant effect upon the initial influx, neither has the addition of 1 mM β-alanine, hypotaurine, or GABA. Consequently, a small fraction (about 20%) of the total taurine uptake is Na^+-dependent but differs from the β-system in being Cl^--independent and taurine selective.

It appears from the above results that 80% of the total taurine uptake at low extracellular taurine concentrations in cells grown in the presence of 1 mM taurine represents unsaturable diffusion. Taurine diffusion is in other cells via volume-activated channels characterized by their sensitivity to inhibitors of anion transport e.g. DIDS and MK196 (see Lambert and Hoffmann, 1994; Strange and Jackson, 1995). Figure 4 shows the effect of DIDS (0.3 mM, closed circles) and MK196 (1 mM, closed triangles) on the cellular taurine content as a function of time after addition of ^{3}H-taurine. The open symbols represent the respective values from parallel controls with no inhibitors added. It is clearly seen that DIDS (Fig. 4, closed circles) as well as MK196 (closed triangles) strongly inhibits taurine influx in *Tetrahymena*. A dose-response relationship was established for the inhibitory effect of DIDS and MK196 on the initial taurine influx (Fig. 5). Although the concentration-dependence of the two drugs seems to be slightly different, DIDS having a more pronounced effect in the low concentration range than MK196, both drugs inhibit the initial influx by nearly 80% at 1 mM. The IC_{50} is estimated from Fig. 5 at 0.6 and 0.8 mM for DIDS and MK196, respectively, and is thus in the same concentration range. The apparent difference between the effect of DIDS and MK196 shown in Fig. 4 is, therefore, due to the difference between the concentrations used. Assuming that the saturable component (20% of the total taurine uptake) is *insensitive* to inhibitors of anion transport,

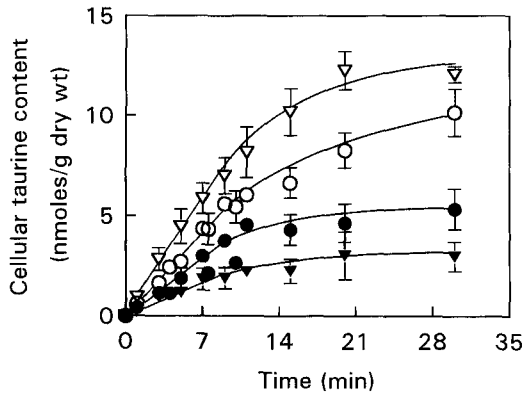

Fig. 4. The effect of DIDS and MK196 on taurine uptake by cells cultivated in the presence of 1 mM taurine. The inhibitors were added 2 min before ^{3}H-taurine (3.7 kBq·ml^{-1}, final concentration 1.3 μM). The cultures were otherwise treated essentially as described in the legend to Fig. 2. DIDS (0.3 mM): ●; MK196 (1 mM): ▼. The equivalent open symbol represent the respective controls. The figure represents 5 experiments with DIDS and 3 with MK196

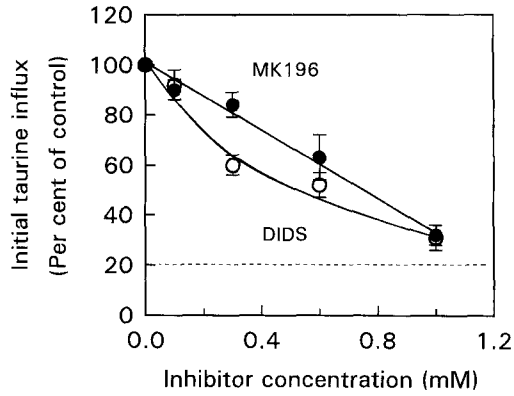

Fig. 5. Dose-effect relationship for DIDS and MK196 on the initial taurine influx in cells grown with added taurine. Inhibitors were added 2 min prior to ^{3}H-taurine (3.7 kBq · ml^{-1}, final concentration 1.3 μM) and samples were subsequently removed for the determination of the initial influx. The influx (in nmoles · g dry wt^{-1} · min^{-1}) was calculated by linear regression of measurements obtained within the initial 7–10 min after addition of the isotope. The measurements represent average ± SEM of 3–11 individual experiments

a maximum inhibition of 80% is to be expected (indicated by the dotted line in Fig. 5). This seems in the case of DIDS and MK196 to be achieved only at concentrations above 1 mM.

Since cultivation of *Tetrahymena* in the presence of taurine increases taurine uptake and since the major component of the initial influx is diffusion in these cells as well as in control cells grown without taurine (see above), it was of relevance to compare the effect of DIDS on taurine influx in cells grown in the presence of taurine (1 mM, 24 hrs) with the effect of DIDS on the influx in cells grown without added taurine. The result is shown in Table 3. In spite of the fact that the total influx is increased by a factor 1.9 in cells grown in the presence of taurine (see above), the DIDS-sensitive fraction of the influx does not differ significantly among the two types of cells.

Aspects of regulation of taurine transport

It has recently been demonstrated that forskolin, which is known to stimulate the adenylate cyclase (Laurenza et al., 1989), increases the cAMP content of Ehrlich cells and causes stimulation of active taurine influx via the β-system (Mollerup and Lambert, 1996). In contrast, the forskolin analogue 1,9-dideoxyforskolin (DDF), which is reported not to affect the adenylate cyclase and thereby the cAMP level (Laurenza et al., 1989; Keogh et al., 1992), is in other cell systems used as an inhibitor of leak fluxes (see e.g. Diaz et al., 1993; White et al., 1992; Shalinsky et al., 1993; Strange and Jackson, 1995). This also includes inhibition of taurine leak fluxes (Kirk and Kirk, 1993; ibid.). Accordingly, the effect of forskolin and DDF on taurine transport by *Tetrahymena* was investigated, and assuming a role for cAMP in stimulation of taurine uptake, the following experiments were conducted using cells grown without added taurine. Figure 6 depicts the effect of 0.1 mM forskolin and 0.1 mM

Table 3

	Initial taurine influx	
	Total influx (relative values)	DIDS-sensitive fraction (per cent of total influx)
Growth without taurine	1	36 ± 5 (n = 14)*
Growth with 1 mM taurine	1.9 ± 0.1 (n = 11)	40 ± 5 (n = 8)*

Tetrahymena cultures were cultivated and transferred to inorganic medium (IMP) as described in Materials and methods. Taurine influx was initiated by addition of ^{3}H-taurine (3.7 kBq · ml^{-1}) and samples were subsequently removed at the desired time points. DIDS (0.3 mM) was added 2 min prior to ^{3}H-taurine. The initial influx was calculated by linear regression of measurements obtained within the first 7–10 min after addition of ^{3}H-taurine. Due to day-to day variations in the absolute influx values the fluxes are given as relative values. Controls and treated cultures were in all cases run in parallel. The concentration of taurine in the medium during the influx measurements was 1.1 μM and 1.3 μM in cultures cultivated without and with added taurine, respectively. This concentration difference was corrected for as described in legend to Fig. 2. *The values are not significantly different.

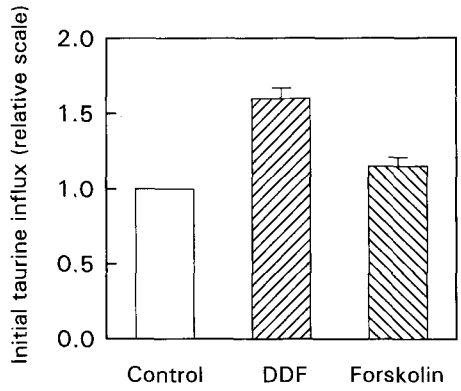

Fig. 6. Effect of forskolin and DDF on the initial taurine influx in cells cultivated without addition of taurine. Forskolin (0.1 mM) and DDF (0.1 mM) were added just prior to ^{3}H-taurine (3.7 kBq · ml^{-1}, final concentration 1.1 μM). The influx was calculated as described in the legend to Fig. 5 and is given as relative values. Controls and experimental cultures were always run in parallel. The figure represents 6 experiments with forskolin and 9 with DDF

DDF, respectively, on the initial taurine influx in cells grown without added taurine. Forskolin seems to increase the initial influx slightly (by 15%), but this increase is not significant. In contrast, DDF increases the influx significantly and by a factor 1.7. From studies with other cell types it is known that DDF interacts with anion transporting systems (see e.g. Diaz et al., 1993), and since taurine leaks in some cell types seem to be mediated via anion channels (Strange and Jackson, 1995), the effect of DIDS on the DDF-induced increase in the initial taurine influx was investigated. Figure 7 shows a typical experiment, where cells were treated in parallel with either DDF, (0.1 mM), DIDS

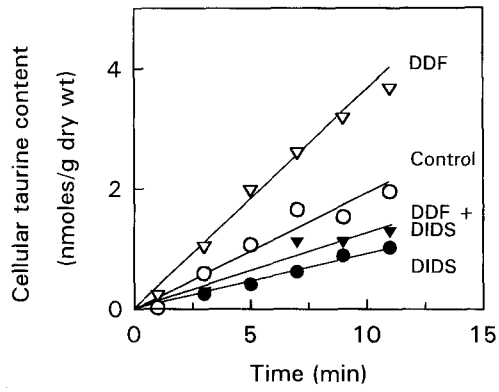

Fig. 7. The effect of DDF and DIDS on taurine influx in cells cultivated without added taurine. DIDS (0.3 mM) was added 2 min prior to the taurine (see above). The figure is representative of 4 separate experiments. Control: ○; DDF; ▽; Control with DIDS: ●; Cells with DDF plus DIDS: ▼

Table 4. The effect of DDF and DIDS on the initial ^{3}H-taurine influx in *Tetrahymena*

	Initial influx (Relative to untreated control)	DIDS-sensitive influx
Control	1	
DDF	1.67 ± 0.08 (7)	
Control with DIDS	0.64 ± 0.04 (14)	0.36
DDF plus DIDS	0.73* ± 0.12 (4)	0.94

Cultures grown in the absence of added taurine were treated essentially as described in legend to Fig. 7. The initial influxes were calculated by linear regression within the first 7–10 min after addition of the labelled taurine. The number of experiments are given in parenthesis. *The influx in cells treated with DDF plus DIDS is 42 ± 4% of the influx in the presence of DDF alone. Hence, the influx in cells treated with DDF plus DIDS is 42% of 1.67 which is 0.73.

(0.3 mM), or DIDS plus DDF in combination. The control cells are represented by open circles. It is seen that DIDS completely blocks the stimulatory effect of DDF on the initial influx (compare open triangles with closed triangles in Fig. 7). MK196 also completely prevents the DDF induced taurine influx (data not shown). Results like the ones shown in Fig. 7 are summarized in Table 4. From Table 4 it is also seen that DDF increases the total taurine influx by a factor 1.7, whereas the DIDS-sensitive portion of the influx is increased by a factor 2.6 by DDF. Thus, DDF seems to activate a DIDS- and MK196-sensitive taurine leak pathway in *Tetrahymena*.

As mentioned above, it is reported that DDF inhibits various anion channels (see e.g. Diaz et al., 1993; Strange and Jackson, 1995). In agreement with this it was reported that the apparent stimulation of vinblastine influx by DDF observed in a multidrug resistant MDR cell line in fact was a result of an inhibition of the efflux (Shalinsky et al., 1993). Consequently, the effect of DDF on the efflux of ^{3}H-labelled taurine from *Tetrahymena* was investigated in cells preloaded with ^{3}H-taurine plus 1 mM non-radioactive taurine for 24 hrs. In separate experiments the efflux of taurine was recorded for a period of at least 10 min. From these experiments it was found that the cellular ^{3}H-activity (cpm·g dry wt^{-1}) was not significantly different before and after transfer of the cells to the inorganic medium. Hence, taurine is not lost from the cells during the washing procedure. The intracellular taurine concentration in cells grown in the presence of 1 mM taurine is, therefore, at the initiation of the efflux experiments still close to 1 mM. Using this value for the initial taurine concentration, the initial cellular ^{3}H-activity, and the loss in cellular ^{3}H-activity (cpm·g dry wt^{-1}·min^{-1}), the initial taurine efflux in the controls was calculated at 61 ± 13 nmoles·g dry wt^{-1}·min^{-1} (n = 4). The effect of DDF

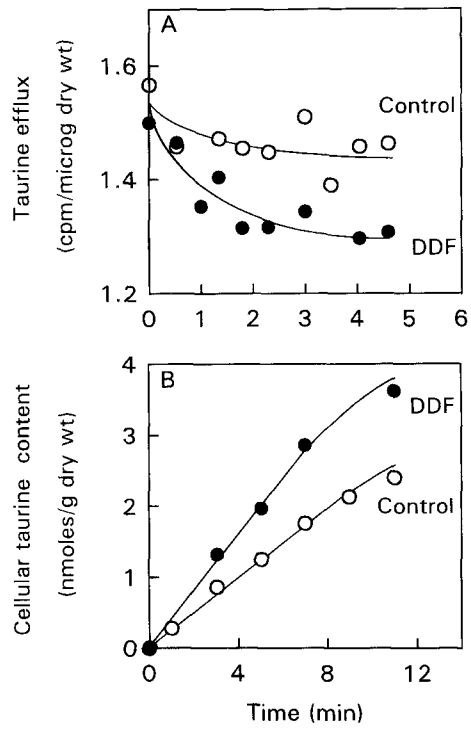

Fig. 8. The effect of DDF on taurine efflux compared to the effect on the influx. **A** Cells were cultivated for 24 hrs in the presence of 1 mM taurine plus ^{3}H-taurine (10 kBq·ml^{-1}) and subsequently transferred to inorganic medium without added taurine as described in Materials and methods. DDF (0.1 mM) was added immediately after the first samples to one of the cultures (closed circles). The panel is representative of 4 experiments. **B** Influx of taurine in cells grown in the absence of added taurine. The experiment was conducted essentially as described in the legend to Fig. 7. The panel is representative of 9 experiments

on taurine efflux is shown in Fig. 8 (panel A), which depicts the result of a typical taurine efflux experiment in which the efflux of taurine was followed with time in the absence and in the presence of DDF, respectively. As seen, the efflux is initially clearly stimulated by DDF (Fig. 8, closed circles). The stimulation seems, however, to be transient. For comparison the effect of DDF on the taurine *influx* is shown in panel B. From Fig. 8 it is evident that taurine efflux is accelerated during the same time interval in which a significant stimulation of the influx is measurable. Thus, DDF seems in *Tetrahymena* to activate a transport pathway which facilitates influx as well as efflux, i.e. a taurine channel.

In Ehrlich cells it has previously been demonstrated that addition of the fatty acid arachidonic acid increases the volume activated taurine channel leak flux seen after hyposmotic cell swelling (Lambert and Hoffmann, 1994). Figure 9 shows the effect of arachidonic acid (0.2mM) on the initial taurine influx in *Tetrahymena* grown without added taurine. From Fig. 9 (panel A) it is seen that arachidonic acid causes an instantaneous dramatic increase in the initial taurine influx. In average the influx is increased by a factor 2.1 ± 0.3

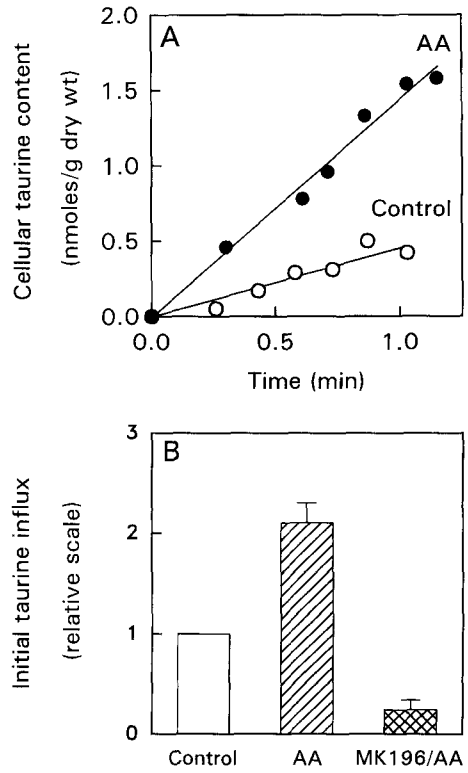

Fig. 9. The effect of arachidonic acid and MK196 on taurine influx in cells cultivated without added taurine. **A** Arachidonic acid (0.2mM) was added immediately prior to the radioactive taurine (see above) and samples were subsequently removed for the determination of radioactivity in the cellular acid soluble pool. The panel is representative of 3 separate experiments. **B** MK196 was added 2min prior to arachidonic acid and taurine. The experiment was otherwise conducted as described for panel A. The experiment is representative of 3 separate experiments

(n = 4). The effect of arachidonic acid on the initial taurine influx is practically abolished by MK196 as shown in Fig. 9 (panel B). In contrast to the effect of DDF, arachidonic acid had apparently no effect on taurine efflux (data not shown). It should be noted that the stimulatory effect of arachidonic acid on taurine influx in *Tetrahymena* is transient. After about 5 min, the uptake rate is reduced and uptake of taurine proceeds again at a rate similar to that seen in the parallel control (data not shown). The results shown in Fig. 9 confirms the notion that taurine channels similar to those found in Ehrlich cells can be demonstrated in *Tetrahymena*.

Discussion

Taurine is taken up by *Tetrahymena* by diffusion as well as by a saturable system (Figs. 2 and 3). In many mammalian cells taurine functions as an osmolyte (Huxtable, 1992). A role for taurine in osmoregulation in *Tetrahymena* is highly improbable due to the low amount of taurine in the cytosol (Scherbaum et al., 1959). *Tetrahymena* is, however, potentially subjected to cell swelling because the cells are hypertonic compared to the surrounding medium (Dunham and Kropp, 1973). The continuos influx of water is compensated for by the activity of the contractile vacuole, which expels excess water and solutes. Thus, transport of water and solutes via active systems as well as via leak systems (channels) must play an important role in the cell volume homeostasis also in *Tetrahymena*.

In spite of the fact that active amino acid transport has been demonstrated in *Tetrahymena* (Aomine, 1981; Hoffmann and Rasmussen, 1972) the observation that the taurine gradient attained after growth in the presence of taurine over night is close to 1 (see Results) makes it highly unlikely that taurine is transported actively during growth in the complex nutrient medium. However, when taurine transport is studied in such *Tetrahymena* cultures following a starvation period of 30 min in an inorganic medium, the initial influx is almost doubled compared to the influx measured in cells grown without added taurine (Fig. 2). Furthermore, the uptake occurs against an apparent significant concentration gradient of about 2. This suggests that part of the uptake occurs via an active system. In agreement with this, a saturable component corresponding to 20% of the total uptake was revealed in the kinetic study shown in Fig. 3. It might, therefore, be speculated that growth in the presence of taurine results in the formation of taurine transporters, which are not expressed unless a starvation period is imposed on the cells.

A small but significant portion of the taurine influx in *Tetrahymena* is Na^+-dependent, Cl^--independent and not inhibitable with structural analogues to taurine (Table 2). Since the Na^+-dependent fraction of the initial influx is of similar magnitude as the saturable component we assume that this represents the active component of the taurine transport in *Tetrahymena*. Thus, the saturable, concentrative taurine uptake in *Tetrahymena* seems to occur via a system which is specific to taurine in contrast to the β-amino acid transporting system seen in mammalian cells, which is Na^+-dependent but also Cl^--

dependent and significantly inhibited by β-alanine, GABA, and hypotaurine (Huxtable, 1992).

In Ehrlich cells active taurine transport, i.e. taurine influx via the β-system is stimulated by cAMP (Mollerup and Lambert, 1996). *Tetrahymena* contains cAMP and all the related enzymes including adenylate cyclase, which is the specific target of forskolin (Ramanathan and Chou, 1973; Laurenza et al., 1989). However, forskolin, which increases the cAMP level in many cells including Ehrlich cells (Laurenza et al., 1989; Mollerup and Lambert, 1996), failed to increase taurine transport in *Tetrahymena* cells grown without added taurine (Fig. 6) as well as in cells grown in the presence of added taurine (data not shown). Since active taurine transport only represents a small fraction of the total taurine influx, a small forskolin-induced increase in the influx might be obscured. However, on the basis of the present observations it seems reasonable to suggest that cAMP and cAMP-mediated phosphorylation are not directly involved in the control of taurine uptake in *Tetrahymena*. This supports the notion that saturable taurine transport in *Tetrahymena* is mediated by a system different from the β-system known from mammalian cells, e.g. Ehrlich cells. It should be noted that cells grown without added taurine also exhibit Na^+-dependent taurine transport and of similar magnitude as that found in cells after growth in the presence of taurine (about 20% of the total uptake, data not shown). This means that growing cells in the presence of taurine increases both the saturable (Na^+-dependent) uptake as well as taurine diffusion.

The above interpretation of the results from Figs. 2 and 3, which lead to the suggestion that 80% of the initial taurine influx in *Tetrahymena* is via a leak pathway, implies that the cellular taurine pool is easily accessible. Thus, taurine efflux from cells grown in the presence of 1 mM taurine, where $[Tau]_i \cdot [Tau]_o^{-1}$ is close to 1, should equal taurine influx at $[Tau]_o = 1$ mM. As stated in "Results", taurine efflux is about 60 nmoles·g dry $wt^{-1} \cdot min^{-1}$. Taurine influx at $[Tau]_o = 1$ mM is 231 ± 30 nmoles·g dry $wt^{-1} \cdot min^{-1}$ ($n = 4$, see Fig. 3). Assuming that 20% of this is by way of a saturable system, the leak flux should amount to about 185 nmoles·g dry $wt^{-1} \cdot min^{-1}$. The discrepancy between the measured influx and the calculated efflux suggests, therefore, that about $^2/_3$ of the cellular taurine pool is not accessible to transport possibly due to compartmentalisation of taurine in food vacuoles and lysosomes during the growth period. The size of the "free" taurine pool at $[Tau]_o = 1$ mM cannot be precisely determined. However, the above calculations suggest that $^1/_3$ of the cellular taurine pool is accessible to transport. Hence, the free cellular pool is expected to be in the magnitude of 300μM in cells grown for 24 hr in the presence of 1 mM taurine. Consequently, the uptake shown in Fig. 2, closed circles, occurs against a much steeper gradient than predicted from the distribution of ^{3}H-taurine between the cellular and extracellular compartments.

Since taurine, due to its zwitterionic nature, is highly water soluble (Huxtable, 1992) diffusion cannot be through the lipid membrane but must be mediated by some sort of channel or by an exchange mechanism. Taurine channels have been demonstrated in numerous cell systems and they are

typically activated by hyposmotic cell swelling and are inhibited by DIDS and MK196 (for references see Lambert and Hoffmann, 1994; Strange and Jackson, 1995). DIDS is usually used as an inhibitor of anion exchange systems but is also reported to affect channels, whereas MK196 is reported to primarily affect anion channels (Chabantchik and Greger, 1992). Taurine channels are, therefore, often reported to represent anion channels (Strange and Jackson, 1995). The existence of taurine channels in *Tetrahymena* with a similar pharmacology as that of the volume activated taurine channels from mammalian cells is confirmed from the effect of DIDS and MK196 on taurine uptake shown in Figs. 4 and 5. Assuming that MK196 does not inhibit exchange systems, the present results are, therefore, taken to indicate that taurine diffusion in *Tetrahymena* occurs via a channel.

In several studies it was reported that the forskolin analogue 1,9-dideoxyforskolin (DDF) also inhibits volume activated taurine channels (see Strange and Jackson, 1995). Our results shown in Figs. 6 and 7 are in striking contrast to this. In *Tetrahymena* DDF causes a stimulation of both influx and efflux of taurine. The DDF-stimulated taurine influx is completely inhibitable with DIDS (Fig. 7, and Table 4). Thus, DDF seems to specifically activate a DIDS-sensitive taurine transport in *Tetrahymena*. It might be speculated that the inhibitory effect of DDF on the volume activated taurine channel in mammalian cells is due to the fact that these channels are only active following stimulation via a complicated cellular signalling system (see Strange and Jackson, 1995) involving among other factors changes in cell volume and thus conformational changes in membrane associated proteins. In *Tetrahymena* the channels are permanently active and no conformational changes of the DDF target are likely to occur prior to interaction of DDF with the cell membrane.

In Ehrlich cells taurine efflux via the volume activated taurine channel is strongly stimulated by arachidonic acid (Lambert and Hoffmann, 1994). Arachidonic acid stimulated taurine influx in *Tetrahymena* (Fig. 9) and the stimulation is completely blocked by MK196 suggesting that arachidonic acid stimulates taurine channels also in *Tetrahymena*. This is in contrast to observations with other cells, where the VSOAC (volume-sensitive organic osmolyte-anion channel) was found to be inhibited by arachidonic acid (Strange and Jackson, 1995). The reason for this discrepancy is not understood. It should be noted that arachidonic acid only seems to stimulate taurine influx in *Tetrahymena* transiently, the effect disappearing about 5 min after addition of arachidonic acid. Furthermore, in contrast to the effect of DDF, arachidonic acid fails to stimulate efflux (data not shown). The reason for this is not clear, but the observations might indicate that arachidonic acid is quickly metabolized or incorporated into the cytoplasmic membrane in *Tetrahymena*.

In conclusion, taurine uptake in *Tetrahymena* comprises a small saturable component and a large diffusional component. The saturable component only resembles the system for β – amino described in vertebrate cells acid transport with respect to its Na^+-dependence. The diffusion pathway seems to represent channels sensitive to inhibitors of anion transport systems. These channels

are, in contrast to the osmolyte channels in other cells, permanently active, and are stimulated by DDF and arachidonic acid.

Acknowledgements

The work was supported by a grant from the Carlsberg Foundation (B. Kramhøft).

References

Aomine M (1981) The amino acid absorption and transport in protozoa. Comp Biochem Physiol 68A: 531–540

Ballentine R, Burford DD (1960) Differential density separation of cellular suspensions. Anal Biochem 1: 263–268

Chabantchik ZI, Greger R (1992) Chemical probes for anion transporters of mammalian cell membranes. Am J Physiol 262: C803–C827

Connolly JG, Kerkut GA (1983) Ion regulation and membrane potential in Tetrahymena and Paramecium. Comp Biochem Physiol 76A: 1–16

Diaz M, Valverde MA, Higgins CF, Rucăreanu C, Sepúlveda FV (1993) Volume-activated chloride channels in HeLa cells are blocked by verapamil and dideoxyforskolin. Pflügers Arch 422: 347–353

Dunham PB, Kropp DL (1973) Regulation of solutes and water in *Tetrahymena*. In: Elliott AM (ed) Biology of *Tetrahymena*. Dowden, Hutchinson, Ross, USA, pp 165–198

Hellung-Larsen P (1988) Parameters affecting the maximum cell concentration of *Tetrahymena*. Experientia 44: 58–60

Hill DL (1972) The biochemistry and physiology of *Tetrahymena*. Academic Press, New York London

Hoffmann EK, Kramhøft B (1969) A relationship between amino acid and sodium transport in *Tetrahymena pyriformis*. Exptl Cell Res 56: 265–268

Hoffmann EK, Rasmussen L (1972) Phenylalanine and methionine transport in *Tetrahymena pyriformis*. Characteristics of a concentrating, inducible transport system. Biochim Biophys Acta 266: 206–216

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72: 101–163

Kaya K, Sano T (1991) Definition of total biosynthesis pathway of taurolipids in *Tetrahymena* cells. Biochim Biophys Acta 1084: 101–104

Kaya K, Uchida K, Kasumi T (1985) Identification of taurine-containing lipid in *Tetrahymena pyriformis* NT-1. Biochim Biophys Acta 835: 77–82

Keogh DP, Mitchell MJ, Crooks JR, Smith SL (1992) Effects of the adenylate cyclase activator forskolin and its inactive derivative 1,9-dideoxyforskolin on insect cytochrome P-540 steroid hydroxylase activity. Experientia 48: 39–41

Kirk K, Kirk J (1993) Volume-regulatory taurine release from a human lung cancer cell line. Evidence for amino acid transport via a volume activated chloride channel. FEBS Lett 336: 153–158

Kramhøft B, Jessen F (1992) Acid extrusion by the ciliate protozoan *Tetrahymena pyriformis*, and activation of a quiescent Na^+/H^+ exchanger by $CuSO_4$. Cell Physiol Biochem 2: 8–17

Kramhøft B, Lambert IH (1995) Taurine uptake under starvation conditions by the protozoan *Tetrahymena pyriformis*. Amino Acids 9: 81

Lambert IH (1984) Na^+-dependent taurine uptake in Ehrlich ascites tumour cells. Mol Physiol 6: 233–246

Lambert IH (1985) Taurine transport in Ehrlich ascites tumour cells: specificity and chloride dependence. Mol Physiol 7: 323–332

Lambert IH, Hoffmann EK (1994) Cell swelling activates separate taurine and chloride channels in Ehrlich mouse ascites tumor cells. J Membrane Biol 142: 289–298

Laurenza A, Sutkowski EM, Seamon KB (1989) Forskolin a specific stimulator of adenylyl cyclase or a diterpene with multiple sites of action? TIPS Rev 10: 442–447

Mollerup J, Lambert IH (1996) Phosphorylation is involved in the regulation of the taurine influx via the β-system in Ehrlich ascites tumor cells. J Membrane Biol 150: 73–82

Ramanathan S, Chou SC (1973) Cyclic nucleotide phosphodiesterase from *Tetrahymena*. Comp Biochem Physiol 46B: 93–97

Saidha T, Stern AI, Schiff JA (1993) Taurine conjugates in the lipid fraction of *Euglena* cells and their mitochondria. J Gen Microbiol 139: 251–257

Scherbaum OH, James TW, Jahn TL (1959) The amino acid composition in relation to cell growth and cell division in synchronized cultures of Tetrahymena pyriformis. J Cell Comp Physiol 53: 119–137

Shalinsky DR, Heath DD, Jehunen AP, Alcaraz JE, Howell SB (1993) Selective modulation of vinblastine sensitivity by 1,9-dideoxyforskolin and related diterpenes in multidrug resistant tumour cells. Br J Cancer 67: 471–479

Strange K, Jackson PS (1995) Swelling-activated organic osmolyte efflux: new role for anion channels. Kidney Intern 48: 994–1003

Wheatly DN, Rasmussen L, Tiedtke A (1994) Tetrahymena: a model for growth, cell cycle and nutritional studies, with biotechnological potential. BioEssays 16: 367–372

White G, Li C, Ishac E (1992) 1,9-dideoxyforskolin does not mimic all cAMP and protein kinase A independent effects of forskolin on GABA activated ion currents in adult rat sensory neurons. Brain Res 586: 157–161

Authors' address: Dr. I. H. Lambert, August Krogh Institute, Biochemical Department, 13, Universitetsparken, DK-2100 Copenhagen Ø, Denmark.

Received May 21, 1996