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Regulation of Taurine Transport in Ehrlich Ascites Tumor Cells

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Summary. Taurine influx is inhibited and taurine efflux accelerated when the cell membrane of Ehrlich ascites tumor cells is depolarized. Taurine influx is inhibited at acid pH partly due to the concomitant depolarization of the cell membrane partly due to a reduced availability of negatively charged free carrier. These results are in agreement with a 2Na,1Cl,1taurine cotransport system which is sensitive to the membrane potential due to a negatively charged empty carrier. Taurine efflux from Ehrlich cells is stimulated by addition of LTD_4 and by swelling in hypotonic medium. Cell swelling in hypotonic medium is known to result in stimulation of the leukotriene synthesis and depolarization of the cell membrane. The taurine efflux, activated by cell swelling, is dramatically reduced when the phospholipase A_2 is inhibited indirectly by addition of the anti-calmodulin drug pimozide, or directly by addition of RO 31-4639. The inhibition is in both cases lifted by addition of LTD_4. The swelling-induced taurine efflux is also inhibited by addition of the 5-lipoxygenase inhibitors ETH 615-139 and NDGA. It is concluded that the swelling-induced activation of the taurine leak pathway involves a release of arachidonic acid from the membrane phospholipids and an increased oxidation of arachidonic acid into leukotrienes via the 5-lipoxygenase pathway. LTD_4 seems to act as a second messenger for the swelling induced activation of the taurine leak pathway either directly or indirectly via its activation of the Cl^- channels, i.e., via a depolarization of the cell membrane.

Key Words Taurine · 5-lipoxygenase · leukotriene D_4 · prostaglandin E_2 · thrombin · phospholipase A_2

Introduction

Organic osmolytes are implicated in anisosmotic cell volume regulation of animal, plant, bacterial, fungal, and protist cells exposed to anisosmotic environments (*see* Chamberlin & Strange, 1989; Law, 1991). Hypotonically swollen Ehrlich cells recover their cell volume (regulatory volume decrease, RVD) by net loss of KCl, taurine (2-amino-ethanesulfonic acid), glycine, alanine and other small nonessential amino acids (Hoffmann & Hendil, 1976). The decrease in amino acids accounts for approximately 30% of the total decrease in osmotically active substances (Hoffmann & Hendil, 1976). A net loss of taurine during RVD is also found in mammalian astrocytes (Pasantes-Morales & Schousboe, 1988) and in cultured MDCK cells (Olea et al., 1991). Preferential loss of taurine from brain cells after reduction of osmolarity has been demonstrated after reduction of osmolarity by as little as 10 mOsm (Wade et al., 1988). Significant amounts of taurine are also lost from brain cells which are swollen under isosmotic conditions by microperfusion with sodium salts of weak organic acids, indicating that this may reflect a volume-protective response exhibited by cells during hypoxic ischaemia (Solis et al., 1990).

The cellular to extracellular concentration gradient for taurine in Ehrlich cells is dramatically diminished in diluted media, and it is furthermore shown that the increase in taurine in the medium during the regulatory volume decrease is practically equivalent to the loss from the cellular pool (Hoffmann & Lambert, 1983). This indicates that the decrease in the intracellular concentration of taurine caused by transfer to hypotonic conditions is due to a change in taurine transport parameters (Hoffmann & Lambert, 1983).

Taurine is present at high concentrations in Ehrlich ascites cells (Hoffmann & Lambert, 1983). At the prevailing taurine concentration in the ascites fluid (0.01 mM, Christensen, Hess & Riggs, 1954), taurine enters the Ehrlich cells by a single, saturable, oxygen-requiring transport process (Kromphardt, 1963, 1965). At external taurine concentration above 1 mM, taurine entry into Ehrlich cells also takes place by a "nonsaturable" process. This flux is a linear function of the extracellular taurine concentration (Kromphardt, 1963; Christensen & Liang, 1966). The saturable system, designated the β-system by Christensen et al. (1954) is highly Na^+ dependent; it has a high affinity for taurine although its transport capacity is low (Lambert, 1984). The maximal taurine influx via the β-system is not influenced by the external Na^+ concentration while the affinity decreases with decreasing external Na^+ concentra-

tion (Lambert, 1984). Substitution of cellular and extracellular Cl^- with other anions identified the taurine uptake via the β-system as Cl^- dependent (Lambert, 1985).

Kromphardt (1965) has demonstrated an acidic group ($pK_A = 6.2$) attached to some membrane constituent, which is essential for the active uptake of taurine. At pH 7.4 this group would be negatively charged and could well represent an anionic site of the taurine carrier. With two Na^+ ions involved in the taurine uptake (*see* Hoffmann & Lambert, 1983; Lambert, 1984), the anionic carrier (C^-) could load with taurine and two Na^+ ions and then be converted to a cationic form (2Na, C, Tau^+). In this case the membrane potential becomes important for the driving of the free carrier to the outer surface and for driving the loaded carrier to the inner membrane. On the other hand, if Cl^- is also cotransported with Na^+ and taurine in an electrical neutral complex complex (2Na, Cl, C, Tau) then the role of the membrane potential is reduced to its effect on the unloaded carrier. In the present study we therefore investigated the charge translocating step in the Na^+- and Cl^--dependent taurine uptake.

The net loss of taurine during RVD in Ehrlich cells is caused mainly by an increased efflux, presumably by stimulation of a Na^+-independent leak pathway (Hoffmann & Lambert, 1983). During RVD the cell membrane depolarizes (Lambert, Hoffmann & Jørgensen, 1989) and the synthesis of leukotrines increases (Lambert, Hoffmann & Christensen, 1987). The present work was therefore initiated to investigate whether the membrane depolarization and/or the volume-induced increase in leukotriene synthesis are involved in the increase in taurine efflux. We have previously proposed that it is leukotriene D_4 which activates Cl^- and K^+ channels after cell swelling (*see* Hoffmann, Lambert & Simonsen, 1988).

Materials and Methods

Cell Suspensions and Incubation Media

Ehrlich ascites tumor cells (hyperdiploid strain) were maintained by weekly intraperitoneal transplantation in White Naval Medical Research Institute (NMRI) mice. To reduce transfer of infection together with the tumor cells, we washed the cells every three weeks in standard medium before implantation. Cells for experimental use were harvested eight days after transplantation and suspended in a standard medium containing heparin (2.5 IU/ml). The cells were washed by centrifugation (700 × *g*, 45 sec), once with the standard solution and twice in the appropriate experimental solutions. The cytocrit was adjusted to 4–6%.

The standard medium (300 mOsm) had the following composition (in mM): 150 Na^+; 5 K^+; 150 Cl^-; 1 Mg^{2+}; 1 Ca^{2+}; 1 sulfate; 1 inorganic phosphate; 3.3 MOPS (3-(N-morpholino)propanesulfonic acid); 3.3 TES (N-tris-(hydroxy-methyl)-methyl-2-aminoethanesulfonic acid; and 5 HEPES (N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)). The pH was adjusted to 7.4. Hypotonic medium (150 mOsm) was prepared by dilution of the isotonic medium with distilled water containing the buffers alone. In solutions with pH 8.2 or 9.0 the buffers were substituted by 5 mM BICINE (N,N-bis(2-hydroxyethyl)-glycine) and 5 mM TRICINE (N-tris-(hydroxymethyl)-methylglycine. NMDG medium was made by substituting N-methyl-D-glucammonium$^+$ for Na^+. The temperature was kept at 37°C, unless otherwise stated.

Cell Volume Measurements

Cell volumes were measured by electronic cell sizing (Coulter counter, model ZB; Coulter channellyzer, model C 1000) using cell cultures diluted 500 times with filtered media (Millipore, pore size 0.45 μm). The final cell density was 80,000 to 100,000 cells/ml; i.e., a cytocrit of 0.01%. Absolute cell volumes in fl (i.e., 10^{-15} liter) were calculated from the median of the cell volume distribution curves using polystyrene latex beads (diameter 13.5 μm, Coulter Electronics) as standards.

Fluorometric Measurements

Fluorometric measurements were performed in polystyrene cuvettes on a Perkin Elmer LS-5 luminescence spectrometer connected to a Perkin Elmer R 100A recorder and a Perkin Elmer CP 100 graphic printer. The temperature of the cuvette was thermostatically controlled and the cell suspension was continuously stirred by use of a Teflon-coated magnet, driven by a motor attached to the cuvette house. *Membrane potentials* were estimated from the fluorescence intensity of the dye 1,1'-dipropyloxadicarbocyanine ($DiOC_3$-(5)). The dye was added to cell suspensions with a cytocrit of 0.25% at a final concentration of 1.6 μM. Excitation and emission wavelength were 577 nm and 605 nm, respectively, and slit widths were 5 nm. Calibration of the fluorescence signal was performed with the cation ionophore gramicidin using cells suspended in Na^+-free, K^+/choline$^+$ media; i.e., standard medium in which NaCl is replaced by KCl and cholineCl and where the sum of potassium and choline is kept constant at 155 mM. The calibration curve was obtained as a plot of the fluorescence *vs.* the Nernst equilibrium potentials for K after addition of gramicidin (*see* Lambert et al. 1989). *Intracellular pH* was assessed by use of the H^+-sensitive bis-carboxyfluorescein derivative (BCECF) of 2,7-bis-carboxyethyl-5(6)-fluoresceinacetoxymethylester (BCECF-AM). The cell cytocrit was 0.15%. The temperature in the cuvette during pH experiments was 25°C in order to minimize leakage of intracellularly trapped BCECF from the cells (Kramhøft, Lambert & Hoffmann, 1988). Excitation wavelengths were 490 nm and 439 nm, emission wavelength was 525 nm and slit widths were 5 nm. Calibration was carried out in KCl medium using the K^+/H^+ ionophore nigericin for dissipation of the pH gradient across the membrane (intracellular pH = extracellular pH) and using Tris (tris(hydroxymethyl) amino methane) and TES as titrants. The calibration curve was obtained by simultaneous recordings of pH in the cuvette and the fluorescence excitation ratio F_{490}/F_{439}; i.e., the 490 nm excitation measurement divided by the 439 nm excitation measurement. For details *see* Kramhøft et al. (1988) and Lambert (1989). For *intracellular Ca^{2+} concentrations* 40-ml cell suspension (cytocrit 0.4%) in standard medium with 0.2% BSA was loaded with 40 μl

1 mM fura-2-AM for 20 min at 37°C. The cells were washed once with standard medium with 0.2% BSA, once with standard medium and finally resuspended at a cytocrit of 5% in standard medium. The fura-2 fluorescence measurements were performed on 3 ml suspension with a 0.5% cytocrit. The measurements were obtained by rapidly shifting the excitation monochrometer between 340 nm and 380 nm, and measuring the emission constantly at 510 nm (*see* Grynkiewicz, Poenie & Tsien, 1985). At the end of each experiment the cell suspension was centrifuged and the fluorescence of the extracellular medium was measured. This value was subtracted from all measurements. The free Ca^{2+} concentration was calculated from the measurements of the ratio of fluorescence intensities according to the equation:

$$[Ca^{2+}] = K_d \cdot ((R - R_{min})/(R_{max} - R)) \cdot S_{f380}/S_{b380}$$

where K_d is the dissociation constant, 135 nM (Grynkiewicz et al., 1985) and R is the fluorescence ratio at 340 nm and 380 nm excitation. R_{max} and R_{min} are the equivalent fluorescence ratios of fura-2 after addition of digitonin at saturating Ca^{2+} concentrations, and in nominally Ca^{2+}-free medium (with 1 mM EGTA), respectively. S_{f380} and S_{b380} are proportionality coefficients, measured from the fluorescence intensity at 380 nm excitation using calibration solutions containing low concentration of free Ca^{2+} and Ca^{2+} concentrations where the dye is saturated.

Taurine Flux Experiments

In taurine *influx* experiments ^{14}C-labeled taurine (0.167 μCi/ml) was added at time zero to 6 ml suspension (cytocrit 6%) giving a final taurine concentration of 1.8 μM. The uptake was followed with time by successive transferring samples (1 ml) of the cell suspension to preweighed vials and separating the cells from the medium by centrifugation (20,000 × *g*, 60 sec); 100 μl of the supernatants were diluted 10 times with perchloric acid (7% final concentration) and saved for determination of extracellular taurine activity. Excess supernatant was removed by suction and the wet weight of the cell pellet was determined by reweighing the samples. The packed cells were then lyzed in 800 μl distilled water, deproteinized by addition of 100 μl perchloric acid (70%) and centrifuged (20,000 × *g*, 10 min). The supernatant was used for determination of cellular taurine activity and the perchloric acid precipitate was dried (90°C, 48 hr) and used for determination of the cell dry weight (*see* Lambert et al., 1989). Cellular taurine activity (CPM/g cell dry weight) was corrected for trapped extracellular medium using ^{3}H-inulin as marker (Hoffmann, Simonsen & Sjøholm, 1979).

In taurine *efflux* experiments the cell suspensions (cytocrit 6%) were equilibrated with ^{14}C-labeled taurine (0.5 μCi/ml) for 60–100 min. At the end of the pre-incubation period duplicate samples were taken for determination of cellular taurine activity (*see above*). For transference of labeled cells to the efflux medium, 1 ml cell suspension was centrifugated in nylon tubes (i.d. 3 mm) for 1 min at 770 × *g*, the tube was cut 1 mm below the interface between the packed cells and the medium. The packed cells were then injected into 7 ml of the appropriate medium by flushing the small sleeve of nylon tube containing the packed cells with 1 ml of the medium. The efflux was followed with time by serially isolating cell-free efflux medium by centrifugation of 1 ml cell suspension through a silicone oil phase (300 μl: 1 part 20 AR/1 part AR 200). At the end of the efflux experiment, triplicate samples of the efflux suspension were taken for determination of protein using bovine serum albumin as a standard (Lowry et al., 1951). The amount of protein (g/ml medium) was converted to cell dry wt (g/ml medium) using a protein/dry weight ratio of 0.78 (Hoffmann & Lambert, 1983). The initial activity of ^{14}C-taurine in the cellular pool (a_o^c) was converted from (CPM/g cell dry wt) to (CPM/ml medium) by multiplication with the amount of cell dry weight (g/ml medium).

Radioactivity was measured in a liquid scintillation spectrometer (Packard TRI-CARB 460C Liquid Scintillation System) using ULTIMA GOLD™ (Packard) as scintillation fluid.

Calculation of Rate Constants for Taurine Transport

The unidirectional taurine influx (J^{oi}) is the rate constant for taurine uptake, k_e'(min^{-1} · g medium/g cell dry wt) multiplied by the extracellular taurine concentration ([TAU]$_e$: μmol/g medium):

$$J^{oi} = k_e' \cdot [TAU]_e \qquad (\mu\text{mol/g dry wt} \cdot \text{min})$$

k_e' is found as the slope of a plot of a_t^c/a_o^m *vs. time* (*see* Fig. 3, upper frame), where a_t^c is the ^{14}C-taurine activity in the cells (CPM/g cell dry wt) at time t and where a_o^m is the ^{14}C-taurine activity in the medium (CPM/g medium) as *zero time*. Since the time course of ^{14}C-taurine loss from the medium during influx was linear within the first 5 min, we used four to five points, taken within this period, to estimate a_o^m by linear regression fit.

The unidirectional taurine efflux (J^{io}) is the rate constant of taurine efflux, k_c' (min^{-1} · g cell water/g dry wt) multiplied by the cellular taurine concentration ([TAU]$_c$: μmol/g cell water):

$$J^{io} = k_c' \cdot [TAU]_c \qquad (\mu\text{mol/g dry wt} \cdot \text{min})$$

k_c' is found as the product of the cell water content ([H_2O]: g/g cell dry wt) and the slope of a plot of $(a_t^m - a_o^m)/a_o^c$ *vs. time* (*see* Fig. 6), where a_t^m and a_o^m are the ^{14}C-taurine activity in the efflux medium (CPM/ml medium) at time t and *zero time*, respectively, and where a_o^c is the cellular ^{14}C-taurine activity (CPM/ml medium) at *zero time* (*see above*).

Measurements of Ion Content

Potassium and sodium were assessed by atomic absorption flame photometry (Perkin Elmer atomic absorption spectrophotometer, model 2380) using samples prepared as for determination of cellular and extracellular taurine activity (*see above*) and correcting all data for trapped extracellular medium.

Chemicals

Stock solutions of gramicidin (1 mM), nigericin (1 mg/ml), prostaglandin E_2 (PGE_2, 2 mM), quinine hydrochloride (1 M), valinomycin (1.2 mM), nordihydroguaiaretic acid (NDGA, 50 mM), (all from Sigma Chemical, St. Louis, MO), $DiOC_3$-(5) iodide (1.2 mM) (Molecular Probes, Junction City, OR), pimozide (5 mM) (Janssen Biochemica) were prepared in ethanol and kept at −22°C until use. BSA, bovine serum albumine was obtained from Sigma. EGTA (ethylene glycol bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid) was obtained from Sigma and added from a stock solution adjusted to pH 7.2 with Tris. BCECF-AM (1 mg/ml) and fura-2-AM (Molecular Probes) were dissolved in dry dimethyl sulfoxide (DMSO). Leukotriene D_4 (LTD_4) was donated by Dr.

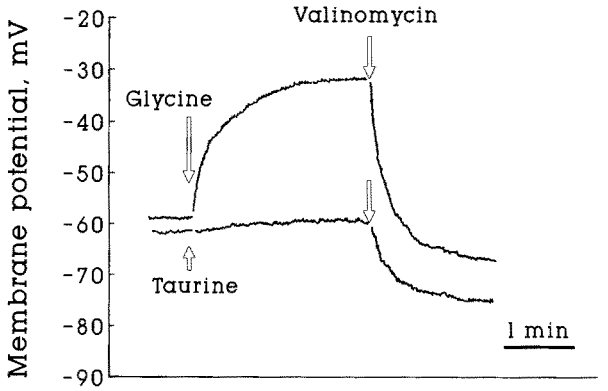

Fig. 1. Effect of glycine and taurine on the cell membrane potential in Ehrlich ascites tumor cells. The potential-sensitive dye $DiOC_3$-(5) was added to Ehrlich cells preincubated in isotonic NaCl medium for 30 min (1.6 μM, cytocrit 0.25%). Taurine (0.5 mM) and glycine (2 mM) was added to min after the dye, at which time the fluorescence signal had become stable. Valinomycin (1.5 μM) was added in order to verify the presence of a steep K gradient across the cell membrane, as seen by the induced hyperpolarization. Absolute potentials were obtained from fluorescence values and a membrane calibration curve as described in Materials and Methods. The curves represent one out of four identical experiments.

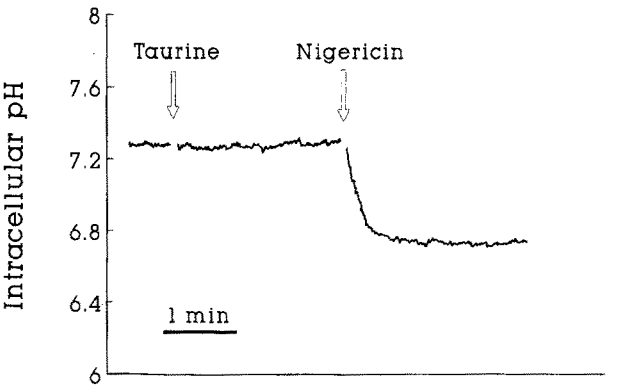

Fig. 2. Effect of taurine on intracellular pH (pH_i) in Ehrlich ascites tumor cells. Ehrlich cells, loaded with the pH-sensitive probe BCECF in isotonic NaCl medium, were diluted with isotonic NaCl medium to a final cytocrit of 0.15%. Excitation wavelengths were 490 nm and 439 nm, while emission wavelength was 525 nm. Taurine (0.25 mM) and K^+/H^+-exchanger nigericin (5 μM) were added as indicated by the arrows. Calibration was carried out in KCl medium as described in Materials and Methods. The figure is representative of three identical experiments.

A.W. Ford-Hutchinson (Merck Frosst, Canada). ETH 615-139, donated by Dr. I. Ahnfelt-Rønne (Løvens Kemiske Fabrik, Copenhagen) and RO 31-4493, RO 31-4639, donated by Dr. D.P. Clough (Roche Product Limited, England), were prepared as stock solutions in ethanol. Silicone oils AR 20 and AR 200 were from Wacker Chemie (Vienna, Austria). ^{3}H-inulin and ^{14}C-taurine were obtained from New England Nuclear.

Results

Taurine Uptake has no Effect on either the Cell Membrane Potential or the Cellular pH

Figure 1 shows the effect of glycine and taurine on the cell membrane potential in Ehrlich ascites tumor cells. As already described by Philo and Eddy (1978) the Na^+-coupled glycine uptake results in a strong depolarization of the cell membrane. In comparison it is seen that taurine has no effect on the cell membrane potential, indicating that taurine uptake is essentially electroneutral. It should be noted that the taurine concentration used (0.5 mM) is eight times the K_m value (0.06 mM) for the taurine uptake system (Lambert, 1984), which thus is saturated. The glycine concentration used (2 mM) is close to the K_m value (2–3 mM) for the glycine uptake system (Kromphardt, 1965; Laris, Pershadsingh & Johnstone, 1976), which is therefore only half saturated.

Figure 2 demonstrates that 0.25 mM taurine has no significant effect on the intracellular pH (pH_i). As a reference we have shown the effect of addition of the K^+/H^+ exchange ionophore nigericin to the cell suspension. The taurine carrier, thus, does not seem to transport any protons.

Effect of the Membrane Potential and the Extracellular pH on the Taurine Uptake System

Figure 3 shows the effect of glycine and quinine on the unidirectional taurine uptake (*upper frame*) and on the rate constant for taurine uptake (lower frame) in Ehrlich cells. Glycine and quinine are both known to depolarize the cell membrane (*see* Fig. 1 and Lambert et al., 1989). In four separate sets of experiments the depolarization was estimated at 31 mV and 44 mV for glycine and quinine, respectively (*see* legend to Fig. 3). The taurine uptake is in both cases strongly inhibited and it is tempting to suggest that the effect induced by glycine and quinine is an effect of the induced depolarization of the cell membrane.

Figure 4 (*upper frame*) shows that taurine uptake increases with increasing extracellular pH, confirming observation previously published by Kromphardt (1965). Kromphardt proposed that the unloaded carrier is negatively charged with a pK value for the anionic site equal to 6.3. The amount of anionic carrier is calculated as a function of the

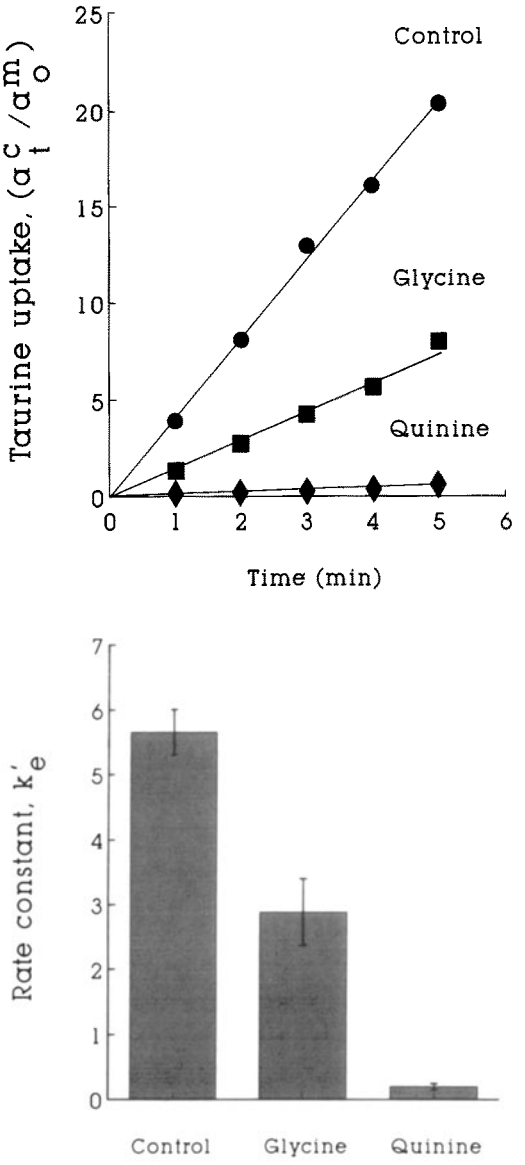

Fig. 3. Effect of depolarization of the cell membrane on the active taurine uptake in Ehrlich ascites tumor cells. Ehrlich cells were pre-incubated for 40 to 60 min in standard NaCl medium. ^{14}C-taurine (0.167 μCi/ml, 1.8 μM, cytocrit 6%) was added at time zero and the cellular ^{14}C-taurine content was followed with time. *Taurine uptake* is given by the relative specific activity, a_t^c/a_o^m, where a_t^c is the cellular ^{14}C-taurine activity at time t (CPM/g cell dry wt) and a_o^m is the initial ^{14}C-taurine activity in the medium (CPM/g medium). *Rate constants* for taurine uptake (k'_e, min^{-1} · g medium/g cell dry wt), are calculated as the slopes of the taurine uptake curves. (*Upper frame*) Taurine uptake after depolarization of the cell membrane. Cells were depolarized by addition of glycine (5 mM) or quinine (1 mM) to the cell suspension two minutes before the addition of ^{14}C-taurine. The resulting membrane potential was in four sets of experiments estimated at -66 ± 2 mV (control cells), -35 ± 2 mV (glycine-treated cells) and -22 ± 2 mV (quinine-treated cells). (*Lower frame*) The rate constants for taurine uptake k'_e after depolarization of the cell membrane are given as the mean ± SEM of three sets of experiments.

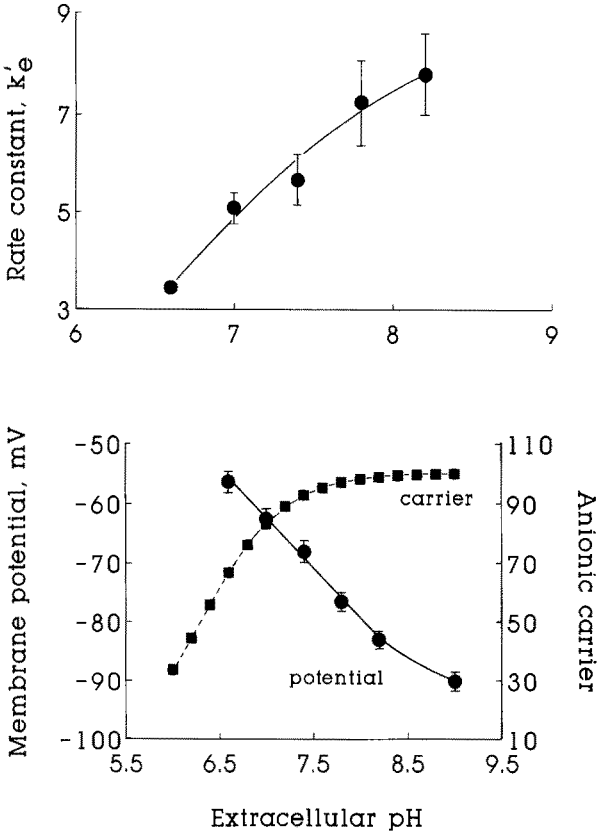

Fig. 4. Effect of extracellular pH on the rate constant k'_e for taurine uptake and the cellular membrane potential in Ehrlich ascities tumor cells. Ehrlich cells, preincubated in standard NaCl medium, pH 7.4 for 30 min, were transferred to standard NaCl medium with variable extracellular pH (pH 6.6, 7.0, 7.4, 7.8, 8.2) and equilibrated for another 15 min. The cellular pH (± SEM) corresponding to extracellular pH 6.6, 7.4, and 8.2, was in 3 separate sets of experiments estimated by the pH-sensitive probe BCECF at 6.90 ± 0.09, 7.38 ± 0.05, 7.76 ± 0.13, respectively. *Rate constants* for taurine uptake (k'_e, min^{-1} · g medium/g cell dry wt) were estimated from the slopes of taurine uptake curves as shown in Fig. 3. *Membrane potentials* (mV) were estimated from the fluorescence of the potential-sensitive dye $DiOC_3$-(5) as shown in Fig. 1. (*Upper frame*) The rate constants for taurine uptake were measured in Ehrlich cells suspended in isotonic NaCl media (1 mM Ca^{2+}) with variable extracellular pH (pH 6.6, 7.0, 7.4, 7.8, 8.2) and given as the mean ± SEM of four independent experiments. (*Lower frame, solid line*) The membrane potentials were estimated in cells suspended in isotonic NaCl media (1 mM Ca^{2+}) with variable extracellular pH (pH 6.6, 7.0, 7.4, 7.8, 8.2, 9.0) and given as the mean ± SEM of five independent experiments. (*Lower frame, dashed line*) The amount (percent) of taurine carrier on anionic form, is calculated from the Henderson-Hasselbach equation assuming a pK = 6.3 for the acidic group of the taurine carrier.

extracellular pH and shown together with measured membrane potentials (Fig. 4. *lower frame*). At high extracellular pH we have simultaneously a highly negative membrane potential and a carrier almost 100% on its anionic form which would accelerate the

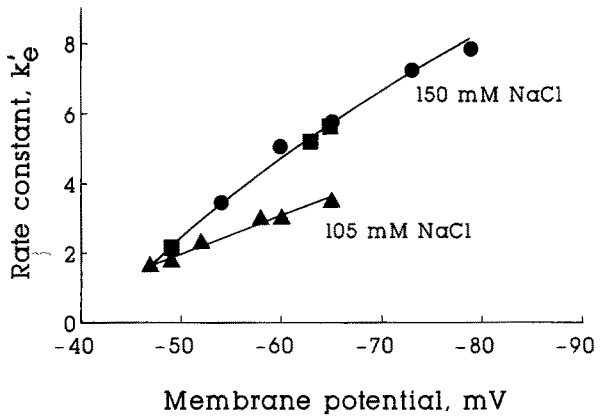

Fig. 5. Effect of cellular membrane potential on the rate constant k'_e for taurine uptake. Cells were equilibrated in NaCl media with variable Ca^{2+} concentrations (*squares*: 1 mM Ca^{2+}, 0.15 mMCa^{2+}, Ca^{2+}-free plus 0.1 mM EGTA), variable pH (*circles*: pH 6.6, 7.0, 7.4, 7.8, 8.2) or variable extracellular K (*triangles*: media with 105 mM NaCl where extracellular K was varied between 5 mM and 50 mM and where the sum of K and N-methyl-D-glucammonium was kept constant at 50 mM). *Rate constants* for taurine uptake (k'_e, $min^{-1} \cdot$ g medium/g cell dry wt) and *membrane potentials* (mV) were estimated as described in the legend to Fig. 4. The data with variable pH are the combined data from the upper and lower frames, in Fig. 4, the data with variable Ca^{2+} are the mean of three experiments, and the data with variable K represent three experiments.

transport of unloaded carrier to the outside of the cell membrane. Such faster return of the carrier could explain the increase in the taurine influx seen at alkaline pH (Fig. 4, *upper frame*). In Fig. 5 the membrane potential has been varied by three different means and the rate constant for taurine uptake is plotted *vs.* the measured membrane potential. The circles are the combined data from the upper and the lower frame in Fig. 4. The results indicated with squares are obtained by varying the extracellular Ca^{2+} concentration and keeping the extracellular pH constant, while the results indicated with triangles are obtained by increasing extracellular K^+. At 1 mM extracellular Ca^{2+} the cell membrane potential is significantly more negative than under Ca^{2+}-free conditions (*see* Table). The Table also demonstrates that intracellular Ca^{2+} is significantly decreased in Ehrlich cells suspended in Ca^{2+}-free medium compared to cells suspended in medium with 1 mM Ca^{2+}. Since Ehrlich cells have a Ca^{2+}-activated K^+ channel (Valdeolmillos, Garcia-Sancho & Herreros, 1986) which almost entirely determines the cell membrane potential (Lambert et al., 1989), it is likely that the depolarization of the cell membrane seen under Ca^{2+}-free conditions is the result of the decrease in intracellular Ca^{2+}. From the data in Fig. 5 it is seen that depolarization induced by variation in extracellular Ca^{2+} or variation in extracellular K^+ in both cases results in a decreased taurine uptake rate. It is noted that the rate constants obtained by increased extracellular K^+ are lower compared to the other rate constants at the same membrane potential because extracellular Na^+ was reduced from 150 mM to 105 mM in order to keep the osmolarity constant and because the taurine influx is strongly Na^+ dependent (Lambert, 1984). These results confirm the hypothesis that the cell membrane potential affects the taurine uptake most likely by accelerating the return of the empty, negatively charged carrier to the outside of the membrane.

The Effect of the Membrane Potential and Cellular pH on the Taurine Efflux

The taurine efflux in Ehrlich ascites tumor cells under isotonic conditions is accelerated when the cell membrane is depolarized. This is seen from Fig. 6 which shows the unidirectional taurine efflux and from Fig. 7 which shows the rate constant for taurine release. The cell membrane was depolarized by addition of glycine, quinine or by omission of Ca^{2+} and hyperpolarized by addition of valinomycin. The effect of the cell membrane potential is most simply explained by the hypothesis that efflux and influx under isotonic conditions predominantly use the same carrier system. Figure 8 (*upper frame*) shows the taurine efflux under isotonic conditions as a function of cellular pH (pH_i). In the range pH_i 7.4 to pH_i 7.8 (pH_o 7.4 to pH_o 8.2) the carrier is on its anionic form and the membrane potential is increasingly more negative (*see* Fig. 4, lower frame). The decrease in the rate of taurine efflux in the pH_i range 7.4 to 7.8 could thus be explained from the suggestion that the empty carrier is predominantly returned to the outside of the membrane (*see above*). In the range pH_i 7.4 to pH_i 6.9 (pH_o 7.4 to pH_o 6.6) a decreasing fraction of the unloaded carrier is on the anionic form and, provided that the anionic carrier is the one with affinity for Na^+ and taurine, this reduced availability of anionic carrier could explain the observed decrease in taurine efflux.

To test whether the high taurine efflux induced by osmotic cell swelling (Hoffmann & Lambert, 1983) is via the carrier system described above or alternatively via a separate leak pathway as previously proposed (Hoffmann & Lambert, 1983; Lambert, 1985), we measured the taurine efflux in hypotonic solution at different pH values (Fig. 8, *lower frame*). The pH dependence of taurine efflux is clearly different under isotonic and hypotonic con-

Table. Effect of Ca^{2+} on taurine gradients and taurine transport in Ehrlich ascites tumor cells[a]

	1 mM Ca^{2+}	Ca^{2+}-free	P^g
E_m[b]	−66 ± 1.6 (5)	−49 ± 1.6 (5)	$P < 0.005$[g]
k'_e[c]	5.7 ± 0.3 (9)	2.1 ± 0.5 (3)	$P < 0.005$[g]
$[Ca^{2+}]_i$[d]	87 ± 2 (3)	70 ± 5 (3)	$P < 0.01$[h]
k'_c[e]	0.31 ± 0.03 (11)	0.44 ± 0.05 (8)	$P < 0.005$[g]
Gradient[f]	673 ± 46 (6)	433 ± 33 (7)	$P < 0.005$[g]

[a] Ehrlich cells were equilibrated in isotonic NaCl media with variable Ca^{2+} concentrations (1 mM Ca^{2+}, Ca^{2+}-free plus 0.1 mM EGTA).
[b] E_m, the membrane potential (mV) is estimated from the fluorescence of $DiOC_3$-(5).
[c] k'_e, the rate constant for taurine uptake (min^{-1} · g medium/g cell dry wt) is estimated from the slope of taurine uptake curves as shown in Fig. 3.
[d] $[Ca^{2+}]_i$, the cellular Ca^{2+} concentration (nM) is estimated as described in Materials and Methods. It is noted that the absolute values should be taken with some precaution as calibration is difficult, especially the estimation of R_{max} and R_{min} is uncertain.
[e] k'_c, the rate constant for taurine efflux (min^{-1} · g cell water/g dry wt) is estimated as the product of the slopes of taurine release curves (*see* Fig. 6) and the cell water content.
[f] The taurine gradients are measured as the steady-state gradients for ^{14}C-taurine (0.5 μCi/ml, 1.8 μM, cytocrit 6%) after 70 to 90 min incubation.
[g] P is the level of significance in a Student's t-test using mean values.
[h] P is the level of significance in a paired Students's t-test.

ditions confirming that a leak pathway different from the active uptake system dominates after cell swelling. The cellular pH values used in the upper and the lower frame are both measured under isotonic conditions. It is previously shown that the acidification seen after transfer to hypotonic solution is slow and only amounts to 0.03 pH units during the first minute (Livne & Hoffmann, 1990) which is the time period for the present measurements.

Activation Mechanism for the Taurine Leak Pathway

From Figure 9 it is seen that the taurine efflux is also activated by cell swelling in the absence of external Ca^{2+} just like it has previously been shown for the activation of the K^+ and Cl^- channels (Hoffmann, Lambert & Simonsen, 1986). The taurine efflux is in isotonic media significantly higher in Ca^{2+}-free media than in Ca^{2+}-containing medium (*see* Table), most probably as a result of the concomitant depolarization of the cell membrane (*see* Table) which stimulates taurine efflux (*see* Fig. 7). In hypotonic media taurine efflux is of similar magnitude in the presence and in the absence of Ca^{2+}—if anything it is higher in the absence of Ca^{2+}.

It has previously been shown that the RVD response in Ehrlich cells following osmotic cell swelling in hypotonic medium is inhibited by the anti-

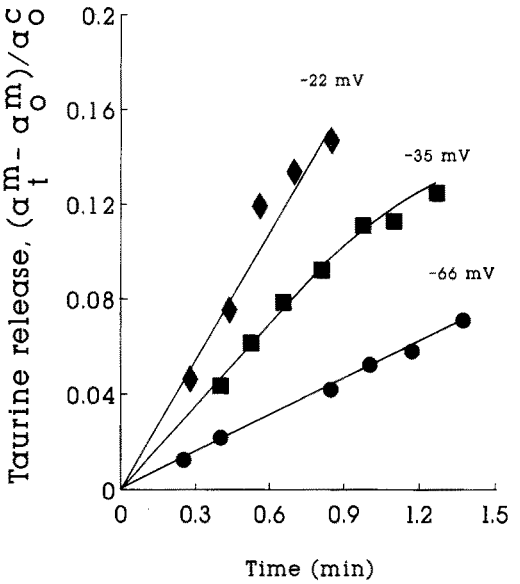

Fig. 6. Effect of the cellular membrane potential on the unidirectional taurine efflux from Ehrlich ascites tumor cells. Ehrlich cells, equilibrated with ^{14}C-taurine (0.5 μCi/ml, 6% cytocrit, 60 min) were at time zero transferred to isotonic NaCl medium. *Taurine release* is shown as the extracellular relative specific activity, $(a_t^m - a_o^m)/a_o^c$ plotted *vs.* the time, where a_t^m and a_o^m are the ^{14}C-taurine activity in the efflux medium at time t and time zero, respectively and a_o^c is the cellular ^{14}C-taurine activity at time zero. *Filled squares, filled diamonds*: cells depolarized by addition of glycine (5 mM) or quinine (1 mM), respectively, to the cell suspension 2 min before the initiation of the efflux experiments. *Filled circles*: control cells. The figure is representative of three experiments.

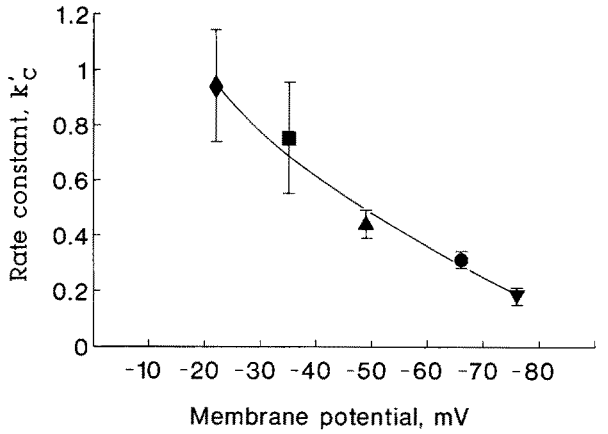

Fig. 7. Effect of membrane potential on the rate constant k'_c for taurine release. Ehrlich cells, preincubated in standard NaCl medium, pH 7.4 with ^{14}C-taurine (0.5 μCi/ml, 6% cytocrit) for 60 min were at time zero transferred to isotonic NaCl medium. The membrane was depolarized by addition of glycine (5 mM, *squares*), quinine (1 mM, *diamonds*), by omission of Ca^{2+} (0.1 mM EGTA, *triangles*) and hyperpolarized by addition of valinomycin (1 μM, *upside-down triangles*). Control cells (*circles*) are cells in standard NaCl medium (1 mM Ca^{2+}). *Rate constants* for taurine efflux (k'_c, min · g cell water/g dry wt) are calculated as the product of the slopes of taurine release curves (*see* Fig. 6) and the cell water content. *Membrane potentials* (mV) are estimated from the fluorescence of the potential-sensitive dye $DiOC_3$-(5) as shown in Fig. 1.

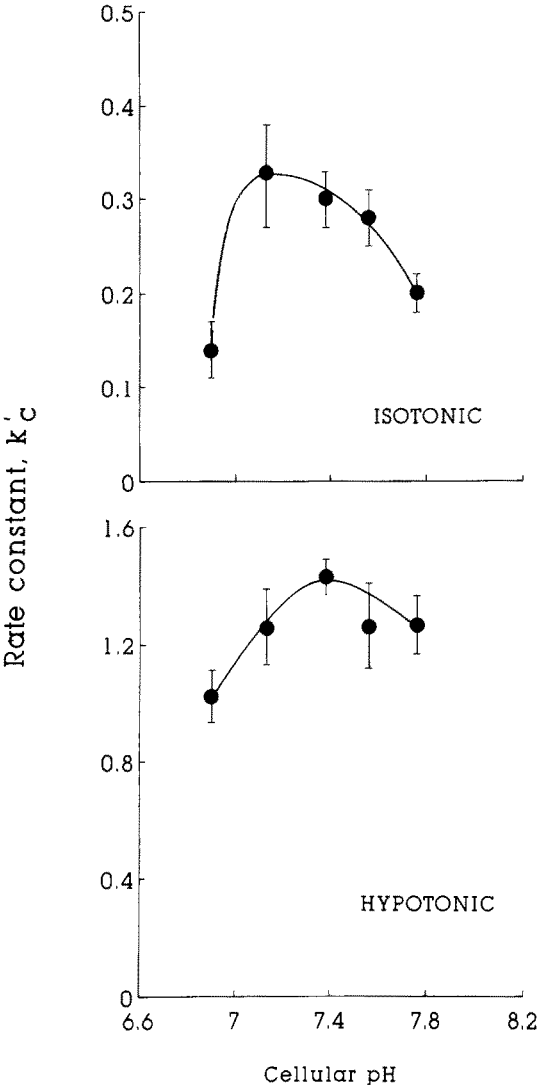

Fig. 8. Effect of cellular pH on the rate constant k'_c for taurine release from Ehrlich cells under isotonic conditions and after swelling in hypotonic media. Ehrlich cells, preincubated in standard NaCl medium, pH 7.4 with ^{14}C-taurine (0.5 μCi/ml, 6% cytocrit) for 60 min were at time zero transferred to isotonic (300 mOsm) or hypotonic (150 mOsm) media, pH 6.6, 7.0, 7.6, 7.8 or 8.2. *Rate constants* for taurine efflux (k'_c, min · g cell water/g dry wt) are calculated as the product of the slopes of taurine release curves (*see* Fig. 6) and the cell water content. *Cellular pH* was measured in separate experiments, using the fluorescent probe BCECF. (*upper frame*) The rate constants for taurine efflux in *isotonic* media are the mean ± SEM of four experiments. (*lower frame*) The rate constants for taurine efflux in *hypotonic* media are given as the mean ± SEM of three experiments.

calmodulin drug pimozide (Hoffmann, Simonsen & Lambert, 1984), by RO 31-4639 (Lambert & Hoffmann, 1991) which inhibits the phospholipase A_2 (*see* Henderson, Chappell & Jones, 1989) and by NDGA and ETH 615-139 (Lambert, Hoffmann & Christensen, 1987; Lambert & Hoffmann, 1991) which inhibit the 5-lipoxygenase (Cashmann, 1985; Kirstein, Thomsen & Ahnfelt-Rønne, 1991). Figure 9 (*middle frame*) demonstrates that these inhibitors also strongly reduce the activation of the taurine leak pathway following cell swelling in hypotonic medium. Since phospholipase A_2 seems to be Ca^{2+}/calmodulin regulated (Van den Bosch, 1980; Craven & DeRubertis, 1983) the effect of pimozide is likely to represent an indirect inhibition of the phospholipase A_2. Thus, inhibition of either phospholipase A_2 or the 5-lipoxygenase prevents the activation of the taurine leak pathway. In the case of ETH 615-139 the taurine efflux is inhibited to the level found in cells suspended in isotonic medium (*compare* middle and upper frame in Fig. 9).

Cell swelling has been found to increase the synthesis of leukotrienes and decrease the synthesis of prostaglandins in Ehrlich cells (Lambert et al., 1987) and addition of LTD_4 is found to activate K^+ and Cl^- channels (Lambert, 1989), whereas PGE_2 activates Na^+ channels (Lambert et al., 1987). Figure 9 (*lower frame*) shows that PGE_2 has no effect on the taurine leak flux whereas LTD_4 has a dramatic activating effect. It has been demonstrated that inhibition of the RVD response in the presence of the anti-calmodulin drug pimozide is lifted by addition

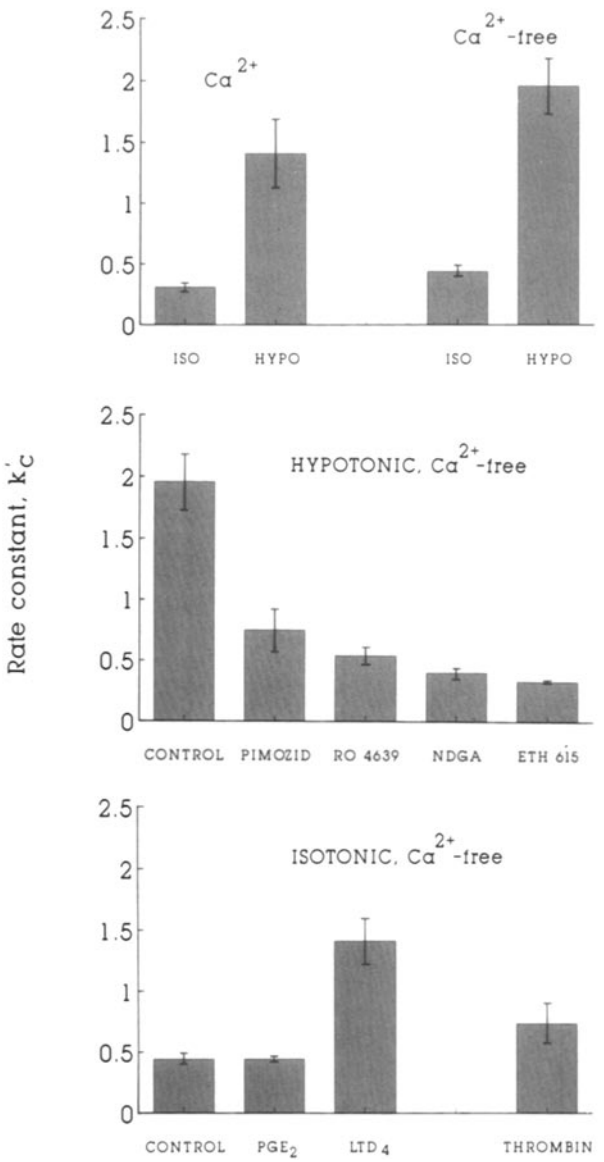

Fig. 9. The effect of extracellular Ca^{2+}, phospholipase-A_2 inhibitors, 5-lipoxygenase inhibitors, PGE_2, LTD_4 and thrombin on the taurine efflux in Ehrlich ascites tumor cells. Ehrlich cells, equilibrated with ^{14}C-taurine (0.5 μCi/ml, 6% cytocrit) for 60 min in standard NaCl medium (pH 7.4, 1 mM Ca) or Ca^{2+}-free NaCl medium (pH 7.4, 0.1 mM EGTA), were at time zero transferred to isotonic (300 mOsm) or hypotonic (150 mOsm) solutions with or without calcium. *Rate constants* for taurine release (k'_c, min · g cell water/g dry wt) were calculated as indicated in the legend to Fig. 7. Pimozide (12 μM), RO 31-4639 (2.4 μM), NDGA (50 μM), ETH 615-139 (10 μM), PGE_2 (50 μM), LTD_4 (4 μM) or thrombin (1 IU/ml) were added at time zero. The rate constants are given by the means ± SEM of at least three sets of independent data.

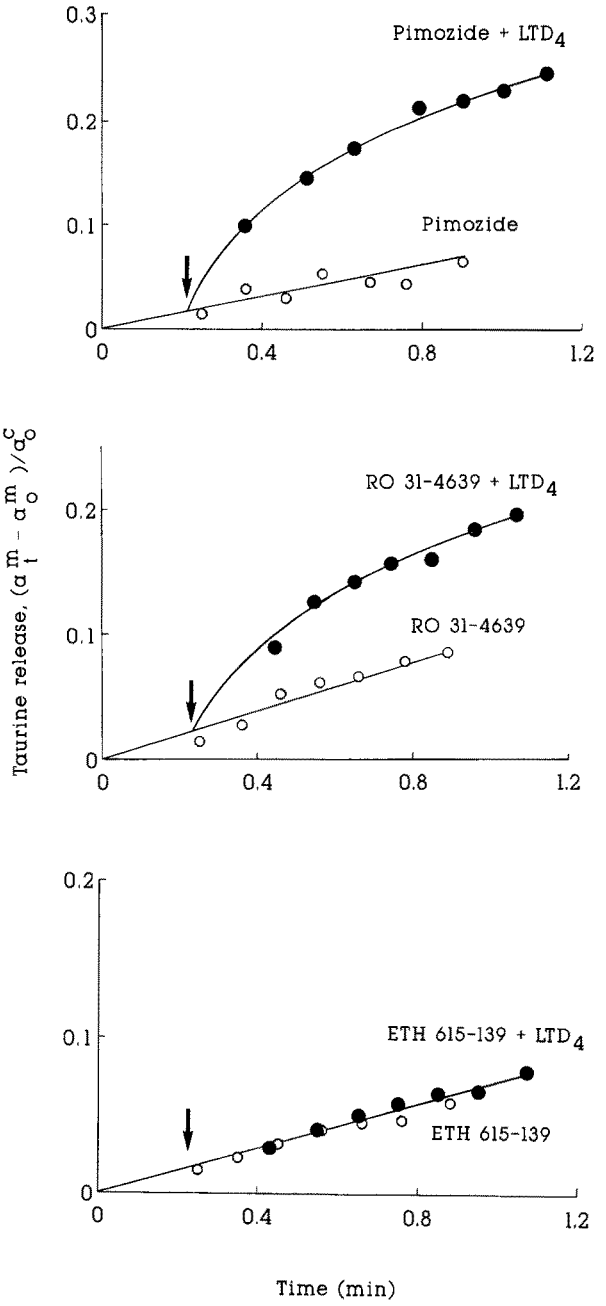

Fig. 10. Effect of LTD_4 on the ^{14}C-taurine efflux when the phospholipase A_2 is blocked by pimozide or by RO 31-4639 and the 5-lipoxygenase is blocked by ETH 615-139. Ehrlich cells, equilibrated with ^{14}C-taurine (0.5 μCi/ml, 6% cytocrit) for 60 min in Ca^{2+}-free NaCl medium (pH 7.4, 0.1 mM EGTA) were at time zero transferred to hypotonic, Ca^{2+}-free NaCl medium with half osmolarity. *Taurine release* is shown as the relative specific activity, $(a_t^m - a_o^m)/a_o^c$ plotted *vs.* the time, where a_t^m and a_o^m are the ^{14}C-taurine activity in the efflux medium at time t and time zero, respectively and a_o^c is the cellular ^{14}C-taurine activity at time zero. Pimozide (12 μM, *upper frame*), RO 31-4639 (2.4 μM, *middle frame*) or ETH 615-139 (10 μM, *lower frame*) were added at time zero. LTD_4 (4 μM, filled symbols) was added at time 0.2 min as indicated by the arrow. Each experiment is representative of at least three similar experiments.

of LTD_4, suggesting a direct effect of LTD_4 on the activation of the K^+ and Cl^- channels which does not involve Ca^{2+} and calmodulin (Lambert, 1989). Figure 10 (*upper frame*) shows a similar effect on

the taurine leak efflux. More recently, it was found that inhibition of the RVD response by phospholipase A_2 inhibitors (Lambert & Hoffmann, 1991) is also lifted by addition of LTD_4, whereas inhibition with the 5-lipoxygenase inhibitor ETH 615-139 is persistent even after addition of LTD_4. In Fig. 10 (*middle and lower frames*) it is demonstrated that the inhibition of the taurine efflux with RO 31-4639 is similarly lifted by addition of LTD_4, while the inhibition with ETH 615-139 is persistent after addition of LTD_4. That the inhibition with ETH 615-139 cannot be lifted by addition of LTD_4 is taken to indicate that ETH 615-139, in addition to the inhibitory effect as a 5-lipoxygenase inhibitor, also acts as an antagonist to the LTD_4 receptor in Ehrlich cells. It has previously been demonstrated that drugs known to act as leukotriene receptor antagonists prevent the effect of LTD_4 in Ehrlich cells (Lambert, 1989). The above results, therefore, support the hypothesis that LTD_4 acts directly on the taurine leak system via binding to a LTD_4 receptor.

Agonists like thrombin and bradykinin, which are known to use inositolphosphates and Ca^{2+} as second messengers are able to mimic the RVD response seen after cell swelling in Ehrlich cells (Simonsen et al., 1990, Hoffmann & Kolb, 1991). Figure 9 (*lower frame*) demonstrates that addition of thrombin to Ehrlich cells also activates the taurine leak efflux significantly ($P < 0.01$, in a Student's *t*-test).

Discussion

Taurine Transport: Electrogenicity and Ion Coupling Ratio

Taurine uptake by killifish renal tubules (Wolff, Perlman & Goldfish, 1986), by vesicles isolated from the basolateral membrane of rat liver cells (Bucuvalas, Goodrich & Suchy, 1987), by brush-border membrane vesicles from rat jejunum (Barnard et al., 1988), rabbit kidney (Wolff & Kinne, 1988) and rat kidney (Chesney et al., 1985; Zelikovic et al., 1989), is stimulated by external Na^+ and Cl^- and by a negative potential on the *trans* side of the membrane. Similarly, the taurine uptake in Ehrlich cells is strongly Na^+ dependent (Lambert, 1984), as well as Cl^- dependent (Lambert, 1985) and a coupling ratio of one Cl^- and two Na^+ to one taurine has been proposed (Hoffmann & Lambert, 1983; Lambert, 1984, 1985). In the present investigation it is demonstrated that taurine influx, when tested under conditions of essential equilibrium exchange where the empty carrier does not contribute to the transport, has no effect on the cell membrane potential (Fig. 1) indicating that taurine influx is electroneutral. On the other hand, taurine influx is stimulated by a negative cell membrane potential (Figs. 3 and 5) and taurine efflux is accelerated when the cell membrane is depolarized (Figs. 6 and 7). These observations are in agreement with a model where the empty taurine carrier is negatively charged and the taurine uptake is an electroneutral 2Na,1Cl,1taurine cotransport.

Kromphardt (1965) has demonstrated that the influx of taurine is reversibly inhibited by H^+ down to a residual, pH-independent fraction. The pH-dependent taurine influx appeared to be proportional to the degree of dissociation of an acidic group with an apparent pK of 6.3, which seemed to be essential to the active taurine uptake although it seemed not to participate directly in the binding of taurine. At pH 7.4 this group is predominantly negatively charged (*see* Fig. 4, *lower frame*) and it is likely that it represents the negatively charged site on the unloaded taurine carrier. Decreasing the extracellular pH will reduce the percentage of unloaded, negatively charged carrier (*see* Fig. 4, *lower frame*) and the cell membrane potential difference will no longer favor diffusion of the unloaded carrier to the outside of the cell membrane. This means that taurine influx is reduced at acid pH (*see* Fig. 4, *upper frame*). The opposite effect is expected for the taurine efflux e.g., a stimulation at decreasing pH values. This is found to be the case in the range pH_i 7.8 to pH_i 7.1 (*see* Fig. 8, *upper frame*). Below pH_i 7.1 (which corresponds to pH_o 7.0 in Fig. 4) the carrier becomes electroneutral and apparently cannot transport taurine. By comparing the slopes in Fig. 5, it can be seen that increasing the influx of taurine by increasing extracellular Na^+ from 105 to 150 mM makes the return of the empty carrier more rate-limiting and thus increases the dependence of the membrane potential. This is in agreement with the hypothesis that the charge of the carrier is an important parameter for taurine transport and that it is the return of the empty carrier which gives the electrogenicity of the taurine transport. For further discussion of this question *see* Heinz, Sommerfeld and Kinne (1988). Furthermore, the observation that taurine uptake is negligible in the absence of Na^+ (Lambert, 1984) indicates that taurine is not transported by the negatively charged carrier alone and that Na^+ must bind to the carrier, changing the tertiary structure and net charge prior to the binding of the taurine molecule. This assumption is supported by the observation that reduction in the extracellular Na^+ concentration reduces the affinity of the transport system for taurine but not the stoichiometry between taurine and the carrier's transport site (Lambert, 1984).

The manner in which Cl^- acts on taurine uptake

is uncertain. In rabbit kidney brush-border membranes (Wolff & Kinne, 1988) Cl^- seems not only catalytically to activate the taurine carrier at a modifier site but also to be transported by the carrier in itself. In the Ehrlich cells taurine uptake is significantly reduced after replacement of Cl^- with more permeable anions such as SCN^- and NO_3^- (Lambert, 1985). This reduced taurine uptake could be explained by the depolarization of the cell membrane resulting from the anion substitution (Lambert et al., 1989) and/or by a low affinity between the taurine carrier and NO_3^- and SCN^-. In the killifish renal tubules Cl^- substitution results in a significant decrease in the V_{max} for taurine uptake whereas the K_m for taurine appears to be unaffected (Wolff et al., 1986), indicating that Cl^- does not increase the affinity between taurine and the taurine carrier. Instead, substituting external Cl^- reduce the Na^+ : taurine transport ratio in the killifish kidney tubules from a 2 : 1 stoichiometry to a stoichiometry closed to 1 : 1 (Wolff et al., 1986). This observation is taken to indicate an effect of Cl^- on the binding of the second Na^+ to the carrier.

In summary, we propose that taurine uptake in Ehrlich ascites tumor cells is a 2Na,1Cl, 1 taurine cotransport, which is driven by the Na^+ gradient. The taurine transport is sensitive to the membrane potential due to the negative charge on the empty carrier. Such a model has previously been proposed for the taurine uptake in rabbit kidney brush-border membranes (Wolff & Kinne, 1988).

It has previously been recognized that Na-dependent transport of neutral amino acids in Ehrlich cells is sensitive to extracellular pH (pH_o). Indeed, one of the distinguishing difference between the Na-dependent (A) and the Na-independent (L) systems is their response to changes in pH. Fluxes via the A-system are strongly reduced by decreased extracellular pH (Christensen, 1962; Kromphardt, 1965) and so are the fluxes via the taurine transporting β-system (Fig. 4, *upper frame*). However, there seems not to be any linkage between movement of H^+ and taurine, because no variation is seen in cytoplasmic pH during taurine uptake (Fig. 2). Furthermore, the H^+ gradient does not seem to be an alternative energy source for accumulation of taurine in Ehrlich cells. This is deduced from Figure 4 (*upper frame*) where it is demonstrated that an increase in extracellular pH from pH 7.4 to pH 8.2 results in a stimulation of the active taurine uptake even though gradient is reversed (pH_i is 7.4 at pH_o 7.4 and pH_i is 7.8 at pH_o 8.2). In accordance, no evidence of a pH gradient-induced change in taurine transport was found in rat renal brush-border membrane vesicles (Chesney et al., 1985; Zelikovic et al., 1989).

Hypotonically swollen Ehrlich ascites tumor cells recover their cell volume (regulatory volume decrease, RVD) by the net loss of KCl, taurine, glycine, alanine and other small non-essential amino acids (Hoffmann & Hendil, 1976). We have previously shown that an increased degradation of amino acids accounts for one third of the cellular alanine loss (Lambert & Hoffmann, 1982), while the loss of taurine, aspartic acid, glutamic acid and glycine is the result of an increased permeability to these compounds (Hoffmann & Lambert, 1983). The initiating signal for the increase in the taurine permeability seems to be the reduction in osmolarity and the stretching of the plasma membrane (cell swelling) and not a result of the reduction in the extracellular ion concentration (Hoffmann & Lambert, 1983).

Since the pH dependence of taurine efflux from Ehrlich cells suspended in hypotonic medium is clearly different from the efflux seen in cells suspended in isotonic medium (Fig. 8) it is assumed that a leak pathway for taurine different from the active taurine uptake system dominates after cell swelling. Evidence for an independent taurine leak pathway has previously been presented (Kromphardt, 1963, Hoffmann & Lambert, 1983, Lambert, 1985).

LTD_4 as a Second Messenger Involved in the Activation of the Taurine Leak Pathway after Cell Swelling

Cell swelling in hypotonic medium is found to be accompanied by a stimulation of the leukotriene synthesis, a reduction in the prostaglandin synthesis (Lambert et al., 1987), and a depolarization of the cell membrane (Lambert et al., 1989). Addition of LTD_4 is known to activate K^+ and Cl^- channels resulting in a depolarization of the cell membrane (Lambert, 1989), whereas PGE_2 stimulates Na^+ channels (Lambert et al., 1987). Figure 9 (*lower frame*) shows that PGE_2 has no effect on the taurine efflux, whereas LTD_4 activates the taurine efflux. The swelling-induced activation of the taurine leak pathway in hypotonic medium is strongly reduced when phospholipase-A_2-mediated release of arachidonic acid from the phospholipids is inhibited either indirectly by addition of the anti-calmodulin drug pimozide or more directly by addition of RO 31-4639 (Fig. 9, *middle frame*). The inhibition is in both cases lifted by addition of LTD_4 (Fig. 10, *upper and middle frame*). Similarly, LTD_4 is able to lift the inhibition of the RVD induced by addition of pimozide or RO 31-4639 (Lambert, 1989, Lambert & Hoffmann, 1991). Inhibition of the 5-lipoxygenase, i.e., inhibition of the oxidation of arachidonic acid into leuko-

trienes by addition of ETH 615—139 or NDGA also blocks the taurine efflux (Fig. 9, *middle frame*) as well as the RVD response after cell swelling (Lambert et al., 1987; Lambert & Hoffmann, 1991). However, the inhibition is not lifted by addition of LTD_4 (Lambert, 1989; Lambert & Hoffmann, 1991), most probably because NDGA and ETH 615-139 in Ehrlich cells also seem to act as antagonists towards the LTD_4 receptor.

In conclusion, it is suggested that the swelling-induced activation of the taurine efflux system and the K^+ and Cl^- channels involves a release of arachidonic acid from the membrane phospholipids and an increased oxidation of arachidonic acid into leukotrienes via the 5-lipoxygenase pathways. LTD_4 seems to act as a second messenger for the activation of the taurine leak pathway either directly or indirectly via its activation of the Cl^- channels; i.e., via the depolarization of the cell membrane.

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