THE Na⁺-INDEPENDENT TAURINE INFLUX IN FLOUNDER ERYTHROCYTES AND ITS ASSOCIATION WITH THE VOLUME REGULATORY TAURINE EFFLUX

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Summary

95% of the Na⁺-independent influx of taurine in flounder erythrocytes at normal osmolality (330 mosmol kg⁻¹) and 0.30 mmol l⁻¹ taurine was mediated by a saturable system (Vₘₐₓ=0.689 nmol g⁻¹ dry mass min⁻¹; Kₘ=0.47 mmol l⁻¹). The influx was inhibited by taurine analogues, but was not significantly affected by reduced osmolality. This saturable influx of taurine was probably mediated by the so-called Na⁺-dependent influx system for taurine operating in the 0 Na⁺: 1 taurine mode. The remaining 5% of the Na⁺-independent influx was mediated by a diffusional pathway (Kₐ₆=0.050 μl g⁻¹ dry mass min⁻¹), since it did not show saturation kinetics, was not inhibited by taurine analogues and did not mediate counter-exchange. This non-saturable influx system for taurine was strongly, but transiently, stimulated by reduction of osmolality. The time course for this stimulatory effect was the same as that for the system that mediates the volume regulatory efflux of taurine. The relative inhibitory effect of bumetanide, furosemide, DIDS and quinine on the fluxes mediated by these two transport systems were also the same. We suggest that these unidirectional fluxes of taurine were mediated by only one transport system: a taurine channel. The effect of reduction of osmolality on the rate coefficient for efflux of β-alanine was equal to the effect on the efflux of taurine, but greater than the effect on the efflux of choline. This difference probably reflects structural and/or electrical restrictions on the substrates to be transported by the taurine channel. The volume regulatory efflux of taurine was inhibited in the presence of the anti-calmodulin drug trifluoperazine and, in a Ca²⁺-free medium, added EGTA. The 5-lipoxygenase inhibitor nordihydroguaiaretic acid completely blocked the volume regulatory efflux of taurine. We suggest that both Ca²⁺/calmodulin and leukotrienes contribute to the control of the transport mediated by the taurine channel.

Introduction

Flounder erythrocytes are capable of maintaining a constant cell volume when the osmolality of the extracellular fluid is reduced (Fugelli and Lange, 1965). This is achieved by a reduction in the amount of certain solutes in the cells. One of these solutes is the amino compound taurine (2-aminoethane sulphonic acid) (Fugelli and

Key words: cell volume regulation, erythrocytes, taurine transport, flounder, Platichthys flesus.
Zachariassen, 1976; Fugelli and Rohrs, 1980; Fugelli and Thoroed, 1986). The shrinkage of cells that follows the initial swelling is often called regulatory volume decrease. During the process of regulation following a reduction of the extracellular osmolality by 75 mosmol l\(^{-1}\) \textit{in vitro}, the content of taurine in the cells is reduced from 68 \(\mu\)mol g\(^{-1}\) dry mass to 34 \(\mu\)mol g\(^{-1}\) dry mass. This loss of taurine represents 30% of the total reduction in the content of solutes in the cells (Fugelli and Thoroed, 1986).

The reduction in the content of taurine is due to net efflux. The rate coefficient for the volume regulatory efflux increases transiently and peaks during the period 20–40 min after the reduction of osmolality. The transport activity in this period is 52 times higher than that before the reduction of osmolality. Thus, the time course for the rate coefficient lags behind the time course for the reduction of the cell volume (Fugelli and Thoroed, 1986). This difference suggests that the volume regulatory efflux of taurine is mediated by a transport system and not by pores generated by passive stretch of the cell membrane. The functional properties of the transport system involved are not yet fully understood. The time course for activation of the taurine transporter also suggests that the transport activity is dependent on cellular signal system(s).

The concentration of taurine in flounder erythrocytes is 200 times higher than in the plasma (S. M. Thoroed and K. Fugelli, unpublished results). The influx of taurine is important for maintaining this concentration gradient. In all types of cells investigated, the uphill transport is mediated by Na\(^+\)-dependent influx systems (see Huxtable, 1992). Na\(^+\)-dependency is also a characteristic of a large fraction of the influx of taurine in flounder erythrocytes (Thoroed and Fugelli, 1993). This influx is apparently mediated by two different influx systems: a system with high affinity (\(K_m=0.013\) mmol l\(^{-1}\) at 145 mmol l\(^{-1}\) Na\(^+\)) and low capacity (\(V_{max}=2.55\) nmol g\(^{-1}\) dry mass min\(^{-1}\)) and another with low affinity (\(K_m=1.34\) mmol l\(^{-1}\)) and high capacity (\(V_{max}=2.55\) nmol g\(^{-1}\) dry mass min\(^{-1}\)) (Thoroed and Fugelli, 1993; see Table 3). In flounder erythrocytes, the influx of taurine is also mediated by Na\(^+\)-independent transport systems (Fugelli and Reiersen, 1978). Little attention has been paid to the Na\(^+\)-independent influx of taurine. This influx, which increases linearly with the extracellular concentration of taurine, has apparently been measured only in order to calculate the fraction of the influx that is Na\(^+\)-dependent. In the present study, we have characterized the Na\(^+\)-independent influx of taurine and examined its connection with the volume regulatory efflux of taurine. We have also studied the effect of different signal systems on the volume regulatory efflux of taurine.

**Materials and methods**

*Reagents and equilibration media*

- \(\beta\)-ABA (\(DL-\beta\)-amino-\(n\)-butyric acid), \(\beta\)-alanine, \(L\)-alanine, 2-aminoethyl phosphonic acid, bumetanide, choline chloride, DIDS (4,4’-diisothiocyanato stilbene-2,2’-disulphonic acid), dimethyl sulphoxide, EGTA, ethanolamine hydrochloride, furosemide, GABA (\(\gamma\)-amino-\(n\)-butyric acid), glycine, homotaurine (3-amino-1-propanesulphonic acid), hypotaurine (2-aminoethanesulphinic acid), nordihydroguaiaretic acid, taurine (2-
aminoethanesulphonic acid), trifluoperazine dihydrochloride and quinine hydrochloride were obtained from Sigma Chemical Co. (St Louis, USA). Aminomethanesulphonic acid was obtained from Janssen Chimica (Beerse, Belgium), N-methyltaurine from Merck (Hohenbrunn, Germany), [methyl-14C]choline chloride and [14C]taurine from Amersham International (Amersham, Great Britain), and β-[14C]alanine from New England Nuclear (Boston, USA). All other reagents were of analytical grade.

The standard incubation media contained the following solutes (mmol l\(^{-1}\)): NaCl, 113 or 145; KCl, 3.20; CaCl\(_2\), 3.75; MgSO\(_4\), 1.25; taurine, 0.30; GABA, 0.30 and Tris, 12.2. HCl was added to obtain pH 7.40 at 10 °C. 330 mosmol kg\(^{-1}\) was chosen as the level of normal osmolality and 255 mosmol kg\(^{-1}\) as the low level. These osmolalities were obtained by adding choline chloride to final concentrations of 43.85 mmol l\(^{-1}\) (330 mosmol kg\(^{-1}\), 113 mmol l\(^{-1}\) Na\(^+\)), 11.85 mmol l\(^{-1}\) (330 mosmol kg\(^{-1}\), 145 mmol l\(^{-1}\) Na\(^+\)) and 3.60 mmol l\(^{-1}\) (255 mosmol kg\(^{-1}\), 113 mmol l\(^{-1}\) Na\(^+\)). Similarly, in Na\(^+\)-free media, in media with various concentrations of taurine and in media to which taurine analogues had been added, normal and low osmolality were maintained by changing the concentration of choline chloride. In media where the concentration of taurine was lower than 0.30 mmol l\(^{-1}\), the concentration of GABA was reduced to the same level. The osmolality was measured using an osmometer (Knauer, Berlin, Germany). The media were aerated for 30 min before the experiments.

Bumetanide, DIDS, furosemide, nordihydroguaiaretic acid, trifluoperazine and quinine were added to the incubation media immediately before use. Bumetanide, DIDS and furosemide were dissolved in dimethyl sulphoxide and nordihydroguaiaretic acid in ethanol. The final concentrations of these solvents in the incubation media were 0.5 % v/v (dimethylsulphoxide) and 0.2 % v/v (ethanol).

**Animals**

Flounders (*Platichthys flesus* L.) (28–45 cm) were caught by gill nets in the inner parts of the Oslo fjord. They were kept at 7–9 °C in aquaria containing sea water (33‰) for at least 7 days before the experiments. They were fed on shrimps during captivity.

**Experimental procedure**

The fish was stunned with a blow in the head and blood was obtained by puncturing the bulbus arteriosus with a heparinized syringe. The blood was transferred to an incubation syringe and the cells were sedimented by centrifugation for 25 s. Each centrifugation was at 3940 g and 8–10 °C (RC2B from Sorvall, Norwalk, USA). The plasma anduffy coat were removed by aspiration. The erythrocytes were then washed four times in medium with normal osmolality (113 or 145 mmol l\(^{-1}\) Na\(^+\)) by resuspension and sedimentation. This washing procedure reduced the number of non-erythrocytes in the cell pellet. Cells from 1–4 fish were used in each experiment. The cells were pooled after the first wash. Following the washing procedure, the erythrocytes were resuspended in medium with normal osmolality and pre-incubated for 120 min in a temperature-controlled water bath (10.0±0.1 °C) (Fugelli and Rohrs, 1980). The medium was renewed twice: after 30 min and 60 min.
Measurement of the influx of taurine

Influx of taurine was measured immediately after the pre-incubation period or at intervals during the following 240 min incubation period. During this period, the media were renewed three times: after 40, 80 and 120 min. Before measuring the influx, the cells were washed three times in their respective tracer-free influx medium. Influx was measured during the following incubation periods of 20 min for the 330 mosmol kg\(^{-1}\) cell samples and 10 min for the 255 mosmol kg\(^{-1}\) samples. The measurements were terminated by centrifugation and resuspension of the cell pellets in tracer-free influx medium. This washing procedure was repeated twice. See Thoroed and Fugelli (1993) for more detailed information. The radioactivity was measured as described by Fugelli and Thoroed (1986). The influx of taurine (nmol g\(^{-1}\) dry mass min\(^{-1}\)) was calculated from the specific activity of \([^{14}C]\)taurine in the influx medium (cts min\(^{-1}\) nmol\(^{-1}\)), the content of \([^{14}C]\)taurine in the cells (cts min\(^{-1}\) g\(^{-1}\) wet mass), the dry mass of the cells (g dry mass g\(^{-1}\) wet mass) and the duration of the incubation period (min). The radioactivity associated with the erythrocytes after 10 min of incubation in Na\(^{+}\)-free medium (355 mosmol kg\(^{-1}\), 320 mmol l\(^{-1}\) taurine, 750 \(\mu\)mol l\(^{-1}\) quinine) at 0 °C (pH 7.40) was considered to be due to non-specific binding and was thus corrected for in the calculations. Quinine was added to block the taurine channels (see Figs 6A and 7).

Measurement of the rate coefficient for efflux

Following the initial washing procedure, the erythrocytes were loaded for 240 min with a radioactive compound (330 mosmol kg\(^{-1}\), 113 mmol l\(^{-1}\) Na\(^{+}\)). The measurements were carried out as described by Fugelli and Thoroed (1986). The rate coefficient for efflux of taurine (\(k_e\), min\(^{-1}\)) was calculated for each of the 20 min periods according to the formula (Caldwell and Keynes, 1969):

\[
k_e = \frac{M_n}{r \left( C_n + \frac{1}{2} M_n \right)},
\]

where \(M_n\) is the content of \([^{14}C]\)taurine in the medium at the end of the \(n\)th sampling period (cts min\(^{-1}\)), \(C_n\) is the total cellular \([^{14}C]\)taurine content at the end of the last sampling period (cts min\(^{-1}\)) and \(r\) is the duration of the sampling period (min).

Measurement of the Na\(^{+}\) concentration

The Na\(^{+}\) concentration (mmol l\(^{-1}\)) was determined by atomic absorption spectrophotometry (SpectrAA-10 from Varian, Mulgrave, Australia). The erythrocytes were haemolyzed in distilled water and the proteins precipitated by the addition of HOCl. Samples of the protein-free supernatant and the incubation media were diluted in CsCl solution (final Cs\(^{+}\) concentration 0.1 % (w/v)). The solution contained Cs\(^{+}\) to suppress ionization of Na\(^{+}\) in the air–acetylene flame.

Statistics

Data were analyzed by Student’s t-test for paired observations. Differences with a \(P\) value of less than 0.05 were considered significant. A non-linear curve-fitting program
Results

The Na⁺-independent influx of taurine: kinetic properties and specificity at normal osmolality

We investigated the kinetic properties of the Na⁺-independent influx of taurine in flounder erythrocytes at normal osmolality (330 mosmol kg⁻¹) by measuring influx at taurine concentrations between 0.024 and 7.5 mmol l⁻¹. The transport activity indicated saturation kinetics in the concentration range 0.024–1.0 mmol l⁻¹ taurine. However, at higher concentrations, the influx apparently continued to increase linearly rather than to approach a plateau. This indicated the existence of more than one Na⁺-independent influx system for taurine. This assumption was confirmed when we plotted the influx values in an Eadie–Hofstee plot (V versus V/[taurine]) (Fig. 1). The line through the data points deviated from linearity.

Non-linear regression analysis was used to investigate both the number and the types of pathways mediating this taurine transport. Earlier investigations had suggested that the Na⁺-independent influx of taurine in flounder erythrocytes is non-saturable (Fugelli and Reiersen, 1978). Therefore, we tested combinations of different numbers of both saturable {V=(V_max[taurine])/(K_m+[taurine])} and non-saturable transport systems (V=K_d [taurine]). Our data were best fitted a model with a single saturable influx system together with a non-saturable system (pseudo r²=0.998). The V_max value of the saturable system was 0.689±0.037 nmol g⁻¹ dry mass min⁻¹ (estimated value ± asymptotic S.D.), while K_m was 0.47±0.06 mmol l⁻¹. The rate coefficient of the non-saturable system (K_d) was 0.050±0.005 µg⁻¹ dry mass min⁻¹.

A Hill plot {log[V/(V_max−V)] versus log[taurine]} of the saturable influx was constructed from the kinetic constants calculated (plot not shown). The Hill coefficient was 1.00, suggesting that if two or more taurine molecules are translocated simultaneously by the transporter, each site of interaction is independent of the other(s).

In studies of Na⁺-independent influx it is important to obtain as low an extracellular concentration of Na⁺ as possible. During the initial washing of the cells in Na⁺-free medium, the extracellular concentration of Na⁺ was reduced from 145 mmol l⁻¹ to 50±11 µmol l⁻¹ (mean value ± s.d.) (N=5). During the influx period, there was a net efflux of Na⁺ from the cells. The content in the cells was reduced by 2.19±0.39 µmol g⁻¹ dry mass, while the extracellular concentration of Na⁺ had increased to 166±47 µmol l⁻¹. Thus, the measurements of the influx of taurine in the present investigation were not made in completely Na⁺-free media. At this final/highest Na⁺ concentration (166 µmol l⁻¹) and with 0.30 mmol l⁻¹ taurine and at normal osmolality the Na⁺-dependent influx of taurine is about 0.084 nmol g⁻¹ dry mass min⁻¹ (Thoroed and Fugelli, 1993). However, this calculated influx represents only about 31% of the saturable influx of taurine measured under the same conditions (Fig. 1). Therefore, the characteristics of the transport systems for taurine demonstrated in this
study are mainly those of the systems that transport taurine independently of extracellular Na$^+$.

The specificity of the Na$^+$-independent influx systems for taurine was examined by measuring influx of [14C]taurine in media with normal osmolality (330 mosmol kg$^{-1}$, 145 mmol l$^{-1}$ Na$^+$, 0.30 mmol l$^{-1}$ taurine), influx of taurine was measured in Na$^+$-free media with unaltered osmolality and various concentrations of taurine between 0.024 and 7.5 mmol l$^{-1}$. Mean values ($N=5$) are shown in an Eadie–Hofstee plot ($V$ versus $V/\text{[taurine]}$). The computer-fitted line (BMDP statistical program for non-linear regression) and the two linear components ($y=-0.469\times10^6x+0.689; x=0.050\times10^{-6}$) are drawn. Pseudo $r^2=0.998$.

The sulphonic acid hypotaurine (52% inhibition) was the only compound that mimicked the inhibitory effect of taurine (53% inhibition) (Table 1). The inhibitory effect of β-alanine was 35%, while the effects of GABA, homotaurine, β-ABA, N-methyltaurine and glycine were low (between 5 and 10%), but significant.

**The Na$^+$-independent influx of taurine: the effect of reduction of osmolality**

In 1978, Fugelli and Reiersen showed that reduction of osmolality stimulates influx of taurine in flounder erythrocytes. Since influx of taurine in these cells is mediated by both Na$^+$-dependent and Na$^+$-independent systems (Fugelli and Reiersen, 1978; Thoroed and Fugelli, 1993), we wanted to investigate whether the Na$^+$-independent systems were affected by reduction of osmolality. Therefore, the Na$^+$-independent influx of taurine was
measured during various intervals in cells readjusting their volume in a Na\(^+\)-containing medium with low osmolality.

Reduction of osmolality strongly stimulated the Na\(^+\)-independent influx of taurine (Fig. 2). During the interval 35–45 min after the reduction of osmolality, the influx of taurine was about 20 times higher than at normal osmolality; 2.50 and 0.12 nmol g\(^{-1}\) dry mass min\(^{-1}\), respectively. During the remaining part of the incubation period, the influx of taurine declined to a level about seven times higher than the level at normal osmolality.

From the results shown in Fig. 2, we could not deduce whether reduction of osmolality stimulated just one or both of the Na\(^+\)-independent influx systems for taurine. Therefore, the kinetics of these transport systems was examined during the interval 230–240 min after the reduction of osmolality. Taurine influx was measured in Na\(^+\)-free media at taurine concentrations between 0.054 and 1.5 mmol l\(^{-1}\) (Fig. 3).

Non-linear regression analysis of the influx values showed that the kinetic constants of the saturable system were not significantly affected by the reduction of osmolality (\(V_{\text{max}}=0.350\pm0.007\) nmol g\(^{-1}\) dry mass min\(^{-1}\); \(K_m=0.52\pm0.01\) mmol l\(^{-1}\)). However, the rate coefficient of the non-saturable system (\(K_d=3.894\pm0.002\ \mu\text{mol g}^{-1}\) dry mass min\(^{-1}\)) was about 78 times higher than at normal osmolality (Fig. 1). Increasing the extracellular concentration of taurine to 105 mmol l\(^{-1}\) resulted in a linear increase of the Na\(^+\)-independent influx (Fig. 4). Higher concentrations of taurine were not tested.

The effect of taurine analogues on the influx of [\(^{14}\)C]taurine at normal osmolality was

<table>
<thead>
<tr>
<th>Compounds tested</th>
<th>Na(^+)-independent influx of [(^{14})C]taurine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Taurine</td>
<td>47.4±13.5(^*) (17)</td>
</tr>
<tr>
<td>(\beta)-Alanine</td>
<td>65.4±8.4(^*) (5)</td>
</tr>
<tr>
<td>Hypotaurine</td>
<td>48.0±12.1(^*) (6)</td>
</tr>
<tr>
<td>GABA</td>
<td>94.8±5.0(^*) (9)</td>
</tr>
<tr>
<td>Homotaurine</td>
<td>91.6±7.3(^*) (11)</td>
</tr>
<tr>
<td>(\beta)-ABA</td>
<td>93.3±4.2(^*) (5)</td>
</tr>
<tr>
<td>(N)-methyltaurine</td>
<td>89.9±4.0(^*) (5)</td>
</tr>
<tr>
<td>Glycine</td>
<td>93.5±3.4(^*) (12)</td>
</tr>
<tr>
<td>l-alanine</td>
<td>100.2±7.2 (5)</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>103.5±7.3 (5)</td>
</tr>
<tr>
<td>Aminomethanesulphonic acid</td>
<td>97.7±9.7 (5)</td>
</tr>
<tr>
<td>2-Aminoethylphosphonic acid</td>
<td>111.1±8.5 (5)</td>
</tr>
</tbody>
</table>

The influx of [\(^{14}\)C]taurine is given as a percentage of the influx of [\(^{14}\)C]taurine in Na\(^+\)-free medium with normal osmolality.

Mean values \(\pm\) s.d. are shown.

The number of experiments is given in parentheses.

\(^*\)Significant inhibition (\(P<0.05\)).

Osmolality-sensitive taurine channels

Table 1. The effect of taurine and taurine analogues on the Na\(^+\)-independent influx of [\(^{14}\)C]taurine in flounder erythrocytes at normal osmolality
tested in the presence of 0.30 mmol l\(^{-1}\) taurine (Table 1). At this taurine concentration, 95 % of the total Na\(^+\)-independent influx was transported by the saturable system. During the interval 230–240 min after the reduction of osmolality, this fraction was reduced to only 10 %. We postulated that only one of the Na\(^+\)-independent systems for influx of taurine was highly specific to taurine. Thus, reduction of osmolality would have a marked influence on the inhibitory effect of taurine analogues on the influx of taurine. This hypothesis was examined by measuring influx of \([^{14}\text{C}]\)taurine in the presence of various taurine analogues (10 mmol l\(^{-1}\)) during the interval 230–240 min after the reduction of osmolality.

None of the compounds tested inhibited influx of taurine (Table 2). The only significant inhibitory effect registered (9 %) was obtained by incubating cells in media where 5 mmol l\(^{-1}\) choline chloride had been replaced by 10 mmol l\(^{-1}\) taurine.

In other types of cells, volume regulatory transport systems for K\(^+\) and Cl\(^-\) are inhibited by furosemide, bumetanide (Kaji, 1986) and/or quinine (Hoffmann et al. 1986; Kaji, 1986). We examined the effect of these compounds on the Na\(^+\)-independent influx...
of taurine during the interval 230–240 min after the reduction of osmolality. Since we have shown that DIDS strongly inhibits the volume regulatory efflux of taurine from flounder erythrocytes (S. M. Thoroed, M. S. Soergaard, E. J. Cragoe and K. Fugelli, unpublished results), we also examined the effect of this compound on the influx of taurine.

In the absence of inhibitors, the Na⁺-independent influx of taurine was 0.92 nmol g⁻¹ dry mass min⁻¹. Each of the compounds tested significantly inhibited this influx. About 75% inhibition was obtained in the presence of DIDS (100 μmol l⁻¹) or quinine (750 μmol l⁻¹) (Fig. 5). The inhibitory effects of furosemide (100 μmol l⁻¹) and bumetanide (100 μmol l⁻¹) were much smaller, about 30% and 15%, respectively. Bumetanide, DIDS and furosemide were dissolved in dimethyl sulphoxide. The final concentration of the solvent in the incubation media was 0.5% (v/v). At this concentration, dimethyl sulphoxide per se had a stimulatory effect (about 5%) on the Na⁺-independent influx of taurine during the period investigated in Fig. 5. Thus, the inhibitory effects of bumetanide, DIDS and furosemide were somewhat greater than that shown in Fig. 5.
The Na\textsuperscript{+}-independent influx of taurine and the volume regulatory efflux of taurine: common properties

Since bumetanide, furosemide and quinine inhibited the osmolality-stimulated influx of taurine (Fig. 5), it was of interest to examine the effect of these compounds on the volume regulatory efflux of taurine.

Quinine (750 \, \mu\text{mol} \text{l}^{-1}) strongly inhibited the volume regulatory efflux of taurine (Fig. 6A). During the period 20–40 min after the reduction of osmolality, the rate coefficient in the absence of quinine was 0.0049 \, \text{min}^{-1}, but it was only 0.0003 \, \text{min}^{-1} in the presence of quinine. Furosemide (100 \, \mu\text{mol} \text{l}^{-1}) (Fig. 6B) and bumetanide (100 \, \mu\text{mol} \text{l}^{-1}) (Fig. 6C), the former more than the latter, also inhibited the volume regulatory efflux of taurine. However, the effects of these two compounds were much smaller than the effect of quinine. Like the Na\textsuperscript{+}-independent influx of taurine, the volume regulatory efflux of taurine was stimulated by dimethyl sulphoxide \textit{per se}. The effect was most pronounced (about 10 \%) in the period 20–40 min after reduction of osmolality. The effect diminished during the rest of the incubation period (data not shown).

In the experiments shown in Fig. 6, exposure of the cells to the various inhibitors started 40 min before the reduction of osmolality. To examine the inhibitory effect of quinine further, the cells were exposed to quinine (750 \, \mu\text{mol} \text{l}^{-1}) during the period 20–80 min after the reduction of osmolality.
Addition of quinine to the incubation medium induced a sharp drop in the rate coefficient for the efflux of taurine (Fig. 7). The maximum inhibitory effect was reached during the subsequent 20 min period. When the quinine-containing medium was replaced by a quinine-free medium, the rate coefficient increased again. During the subsequent 60 min, it reached a peak level which was 83% higher than the maximum value measured in the cells stimulated by reduced osmolality but not exposed to quinine. During the remaining part of the incubation period, the rate coefficient declined towards the level found in the cells not treated with quinine.

Bumetanide, DIDS, furosemide and quinine inhibited both the Na⁺-independent, osmolality-stimulated influx of taurine and the volume regulatory efflux of taurine (Fig. 6; S. M. Thoroed, M. S. Soegaard, E. J. Cragoe and K. Fugelli, unpublished results). An interesting question arising from these results was whether the two fluxes could be mediated by the same transport system.

The relationship between the rate coefficient (k) for a transport process, the flux (J) and the concentration of the substance transported [S] on the cis side of the membrane is given by the equation: $k = J/[S]$. The influx measurements (Fig. 5) were carried out at a constant extracellular concentration of taurine. Thus, the relative effects of the various inhibitors on the rate coefficient for the volume regulatory efflux of taurine were comparable with the corresponding effects on the Na⁺-independent influx. This comparison is shown in Fig. 8. A strong correlation was revealed.

### Table 2. The effect of taurine and taurine analogues on the Na⁺-independent influx of [¹⁴C]taurine in flounder erythrocytes during the period 230–240 min after reduction of osmolality

<table>
<thead>
<tr>
<th>Compounds tested</th>
<th>Na⁺-independent influx of [¹⁴C]taurine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Taurine</td>
<td>91.1±2.7*</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>100.1±8.1</td>
</tr>
<tr>
<td>Hypotaurine</td>
<td>100.6±5.9</td>
</tr>
<tr>
<td>GABA</td>
<td>95.2±8.1</td>
</tr>
<tr>
<td>Homotaurine</td>
<td>99.2±3.3</td>
</tr>
<tr>
<td>β-ABA</td>
<td>95.7±5.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>95.8±4.3</td>
</tr>
<tr>
<td>l-Alanine</td>
<td>106.0±6.0</td>
</tr>
</tbody>
</table>

Following pre-incubation in normal osmolality medium (330 mosmol kg⁻¹, 113 mmol l⁻¹ Na⁺, 0.30 mmol l⁻¹ taurine), the cells were incubated in medium with low osmolality (255 mosmol kg⁻¹, 113 mmol l⁻¹ Na⁺, 0.30 mmol l⁻¹ taurine). The influx media were Na⁺-free media with low osmolality, where some of the choline chloride were replaced isosmotically by 10 mmol l⁻¹ taurine or taurine analogues.

The influx of [¹⁴C]taurine is given as percentage of the influx of [¹⁴C]taurine in Na⁺-free medium with low osmolality.

Mean values ± s.d. (N=5) are shown.

*Significant inhibition (P<0.05).
The hypothesis that these two fluxes could be mediated by the same transport system was further strengthened by comparing the time courses for the Na⁺-independent influx of taurine and the rate coefficient for the volume regulatory efflux of taurine in cells readjusting their volume in Na⁺-containing medium with low osmolality. The two curves were parallel (Fig. 9A).

We have earlier shown that the time courses for the rate coefficient for the volume regulatory efflux of taurine from cells readjusting their volume in Na⁺-containing or Na⁺-free medium with low osmolality differ (Fugelli and Thoroed, 1986). We therefore measured the Na⁺-independent influx of taurine in cells readjusting their volume in Na⁺-free medium during various intervals. The time course obtained was compared with the time course for the rate coefficient for efflux of taurine (Fig. 9B).

The Na⁺-independent influx of taurine was also strongly stimulated when the cells readjusted their volume in Na⁺-free medium with low osmolality. However, after reaching the peak value (about 3.3 nmol g⁻¹ dry mass min⁻¹) during the period 35–45 min, the transport activity declined much more slowly in cells readjusting their volume in Na⁺-free medium (Fig. 9B). In the subsequent interval (115–125 min), the influx of taurine was only 15% lower than the peak value in cells regulating their volume in Na⁺-free media, whereas it was 59% lower in cells incubated in the presence of Na⁺.

During the first 120 min after reduction of osmolality, the time course for the Na⁺-
independent influx in cells readjusting their volume in Na⁺-free medium was parallel to the time course for the rate coefficient for efflux of taurine (Fig. 9B). During the subsequent 120 min, the activity of the two transporters seemed to continue to decline at the same rate. Thus, it seems reasonable to suggest that the Na⁺-independent influx of taurine that is stimulated by reduction of osmolality and the volume regulatory efflux of taurine are mediated by the same transport system.

Figs 3 and 4 show that the influx of taurine that is stimulated by reduction of osmolality is mediated by an apparent non-saturable Na⁺-independent influx system. In addition, Table 2 shows that this system is not inhibited by taurine analogues. These results suggest
that this Na⁺-independent transport of taurine is mediated either by non-saturable pathways or by saturable systems with an extreme capacity, and high specificity, for taurine. In order to distinguish between these two possibilities, we examined the effect of increased extracellular concentrations of taurine on the rate coefficients for efflux of taurine. We also investigated the effect of reduction of osmolality on the rate coefficient for efflux of β-alanine and choline, and compared these effects with the effect on the volume regulatory efflux of taurine.

The effect of increased extracellular concentrations of taurine was investigated in cells readjusting their volume in Na⁺-free media with low osmolality. 120 min after reduction of osmolality, the concentration of taurine was increased from 0.30 mmol l⁻¹ to 75 mmol l⁻¹ or 105 mmol l⁻¹. These changes in the concentration of taurine had no

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Fig. 7. The effect of quinine on the increase in the rate coefficient (min⁻¹) for efflux of taurine from flounder erythrocytes induced by reduction of osmolality. The cells were exposed to quinine during the interval 20–80 min after the reduction of osmolality, as indicated by the bar. (○) 330 mosmol kg⁻¹; (△) 330 mosmol kg⁻¹, 750 μmol l⁻¹ quinine; (△) 330 mosmol kg⁻¹, cells after exposure to 750 mmol l⁻¹ quinine; (●) 255 mosmol kg⁻¹; (▲) 255 mosmol kg⁻¹, 750 μmol l⁻¹ quinine; (▼) 255 mosmol kg⁻¹, cells after exposure to 750 μmol l⁻¹ quinine. The concentration of Na⁺ was 113 mmol l⁻¹ and that of taurine was 0.30 mmol l⁻¹. Mean values ± s.d. (N=4) are shown except where error bars are masked by the symbol. See legend of Fig. 6 for further details.
significant trans-stimulatory effect on the rate coefficient for taurine efflux. At 75 mmol l\(^{-1}\) Na\(^+\), the effect on the rate coefficient was 0.00005±0.00050 min\(^{-1}\) (mean ± S.D., N=3) and at 105 mmol l\(^{-1}\) Na\(^+\), the effect was 0.00003±0.00025 min\(^{-1}\).

The rate coefficient for efflux of β-alanine from flounder erythrocytes was equal to that of taurine, both at normal osmolality and after reduction of osmolality (Fig. 10A). The rate coefficient for the efflux of choline, in contrast, was different (Fig. 10B). At normal osmolality, the rate coefficient declined from 0.0070 min\(^{-1}\) to 0.0023 min\(^{-1}\) during the incubation period. Thus, the activity of the choline transporters was much higher than that of the taurine transporters. The rate coefficient for the efflux of choline was also increased after reduction of osmolality. However, the effect on the efflux of choline was much lower than the effect on the efflux of taurine.

The volume regulatory efflux of taurine: cellular signal systems affecting the transport activity

In order to elucidate the possibility of different signal system(s) contributing to the control of the volume regulatory efflux of taurine, we examined the effect of the anticalmodulin drug trifluoperazine (20 μmol l\(^{-1}\)) (see Weiss et al. 1980) and that of Ca\(^{2+}\)-free medium added 0.80 mmol l\(^{-1}\) EGTA.

The efflux of taurine was significantly inhibited by removing Ca\(^{2+}\)-from the low-osmolality medium (Fig. 11A). The peak value obtained during the period 20–40 min
after the reduction of osmolality was about 22% lower than in cells readjusting their volume in Ca\textsuperscript{2+}-containing medium. The time courses for the rate coefficients were apparently parallel. At normal osmolality, the removal of extracellular Ca\textsuperscript{2+} did not affect the efflux of taurine. A stronger inhibition of the transport activity was observed in erythrocytes readjusting their volume in the presence of trifluoperazine (Fig. 11B). In the absence of trifluoperazine, the peak value was 0.0053 min\textsuperscript{-1}, but it was only 0.0020 min\textsuperscript{-1} in the presence of trifluoperazine.

We also examined the effect of the 5-lipoxygenase inhibitor nordihydroguaiaretic acid (50 \textmu mol l\textsuperscript{-1}) (see Cashman, 1985). Inhibition of 5-lipoxygenase, which catalyzes the initial steps of the conversion of arachidonic acid to leukotrienes, completely inhibited

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**Fig. 9.** The time course for the change in the Na\textsuperscript{+}-independent influx of taurine (nmol g\textsuperscript{-1} dry mass min\textsuperscript{-1}) and the rate coefficient (min\textsuperscript{-1}) for efflux of taurine from flounder erythrocytes following reduction of osmolality from 330 to 255 mosmol kg\textsuperscript{-1}. The cells were readjusting their volume in Na\textsuperscript{+}-containing (113 mmol l\textsuperscript{-1}) (A) or Na\textsuperscript{+}-free medium (B) at 0.30 mmol l\textsuperscript{-1} taurine. Filled symbols, influx; open symbols, efflux; triangles, 330 mosmol kg\textsuperscript{-1}; circles and squares, 255 mosmol kg\textsuperscript{-1}. Mean values ± s.d. are shown. (A) Influx, N=4 (c.f. Fig. 2); efflux, N=31 (c.f. Figs 6, 7 and 11). (B) Influx, N=6; efflux, N=3 (c.f. Fugelli and Thoroed, 1986).
the volume regulatory efflux of taurine (Fig. 11C). Nordihydroguaiaretic acid was dissolved in ethanol. The final concentration of this solvent (0.2% v/v) per se had no significant effect on the volume regulatory efflux of taurine (data not shown).

Thus, these results suggest that both Ca\(^{2+}\)/calmodulin and leukotrienes help to activate the volume regulatory efflux of taurine from flounder erythrocytes.

**Discussion**

*The saturable Na\(^+\)-independent influx of taurine*

About 95% of the Na\(^+\)-independent influx of taurine at 0.30 mmol l\(^{-1}\) taurine and normal osmolality was mediated by a system showing saturation kinetics (\(K_m = 0.47\) mmol l\(^{-1}\); \(V_{max} = 0.689\) nmol g\(^{-1}\) dry mass min\(^{-1}\)) (Fig. 1) and substrate specificity (Table 1). Reduction of osmolality apparently had no effect on this transport activity (Fig. 3). To our knowledge, a Na\(^+\)-independent system for influx of taurine with properties like these has not been demonstrated before. It is an interesting question, however, whether these properties really are those of a separate transport system for taurine. This transport might just as well be only one of the functional modes of the Na\(^+\)-dependent influx system for taurine, here demonstrating its transport activity in the absence of Na\(^+\) (Table 3).

We presume that the last alternative is correct. This hypothesis is based on the following facts. (1) The affinity of the Na\(^+\)-dependent system for influx of taurine is reduced when the extracellular concentration of Na\(^+\) is reduced (Thoroed and Fugelli, 1993). (2) Under similar conditions, the transport capacity of this system is also reduced (Thoroed and Fugelli, 1993). These properties imply that the Na\(^+\)-dependent system is...
able to translocate taurine in the absence of extracellular Na\(^+\) (see Schultz and Curran, 1970). (3) The affinity of the saturable Na\(^+\)-independent system for influx of taurine was lower than that of the Na\(^+\)-dependent system at 0.50 mmol l\(^{-1}\) Na\(^+\), while the transport capacity was in the same range (Figs 1 and 3; Thoroed and Fugelli, 1993). (4) Like the Na\(^+\)-dependent influx of taurine, reduction of osmolality had no significant effect on the saturable Na\(^+\)-independent influx (Fig. 3, Table 3). (5) Like the Na\(^+\)-dependent influx of taurine, substrate specificity was also characteristic of this saturable Na\(^+\)-independent influx of taurine (Tables 1 and 3). Hypotaurine and \(\beta\)-alanine were the most potent inhibitors of both transport processes. The inhibitory effects of the various taurine analogues on the influx of taurine in the nominally Na\(^+\)-free media were much lower.
however, than the corresponding effects on the Na⁺-dependent influx. Probably, this apparent discrepancy was due to the presence of two functional different Na⁺-independent systems for influx of taurine (Figs 1 and 3). The substrate specificity of the saturable system was partly masked by the transport mediated by the second influx system. This latter influx system did not seem to show substrate specificity (Table 2).

The Na⁺-independent system for transport of taurine stimulated by reduced osmolality

The properties of the transport pathway mediating the remaining 5% of the total Na⁺-independent influx of taurine at 0.30 mmol l⁻¹ taurine and 330 mosmol kg⁻¹ (Fig. 1) differed from the properties of the saturable system. (1) It did not show saturation kinetics in the concentration range of 0–105 mmol l⁻¹ taurine (Figs 1, 3 and 4). (2) The transport activity was not significantly inhibited by compounds structurally related to taurine (Table 2). (3) The transport system was transiently strongly stimulated by reduction of osmolality (Fig. 2). In addition, Fig. 5 shows that this Na⁺-independent influx of taurine was inhibited by each of the compounds quinine, DIDS, furosemide and bumetanide. These compounds also inhibit transport systems mediating the volume regulatory efflux of K⁺ from various types of cells (Cala, 1980; Hoffmann et al. 1986; Kaji, 1986). The relative inhibitory effect of each of these compounds on the Na⁺-independent influx of taurine was similar to the corresponding effect on the volume regulatory efflux of taurine (Fig. 8). In addition, the time course for the stimulatory effect of reduction of osmolality on the Na⁺-independent influx of taurine was the same as the time course for its effect on the rate coefficient for the volume regulatory efflux (Fig. 9). These similarities strongly suggest that the two unidirectional fluxes of taurine were mediated by the same transport system.

What kind of transport system mediates these fluxes? We did not demonstrate saturation kinetics in the concentration range 0–105 mmol l⁻¹ taurine (Figs 1, 3 and 4) nor a trans-stimulatory effect from increased influx of taurine on the volume regulatory efflux of taurine. Both properties suggest that the transport of taurine was not carrier-mediated (see Klingenberg, 1989). The apparent lack of substrate specificity for this Na⁺-independent influx of taurine supports this assumption (Table 2). In contrast, the efflux experiments shown in Fig. 10 suggest structural and/or electrical restrictions on the

<table>
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<th>Influx system</th>
<th>$V_{\text{max}}$†</th>
<th>$K_m$†</th>
<th>$K_d$‡</th>
<th>Specificity</th>
<th>Osmolality-sensitive</th>
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<tr>
<td>Low-affinity, high-capacity, Na⁺-dependent*</td>
<td>5.06</td>
<td>1.34</td>
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<tr>
<td>High-affinity, low-capacity, Na⁺-dependent*</td>
<td>2.55</td>
<td>0.013</td>
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<tr>
<td>Saturable, Na⁺-independent</td>
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<td>0.47</td>
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<td>No</td>
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<tr>
<td>Non-saturable, Na⁺-independent</td>
<td>0.05</td>
<td>Low</td>
<td>Yes</td>
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</table>

† The kinetic constants are given as: $V_{\text{max}}$, nmol g⁻¹ dry mass min⁻¹; $K_m$, mmol l⁻¹; $K_d$, $\mu$g⁻¹ dry mass min⁻¹.
substrates to be transported by the system mediating the volume regulatory efflux of taurine. The reduction of osmolality had the same effect on the rate coefficient for the efflux of taurine as on the efflux of β-alanine (Fig. 10A). The time course for its effect on the efflux of choline was also the same as that for taurine, but the effect was much smaller (Fig. 10B).

In the light of the above considerations it seems reasonable to suggest that flounder erythrocytes possess taurine channels. These channels are stimulated by reduction of osmolality, and the passage of taurine through these channels is not dependent on Na⁺. The structural and/or electrical demands on the substrate to be transported by the channel do not appear to be as stringent as the demands on the substrate transported by the Na⁺-dependent system for the influx for taurine (Table 1; Thoroed and Fugelli, 1993).

Taurine helps to maintain a constant volume in a variety of types of cells under hypotonic conditions (see Huxtable, 1992). The kinetics of the pathways mediating the reduction in the intracellular content of taurine have been investigated in only some of these cells. Both influx and efflux of taurine from erythrocytes from Japanese eel (Fincham et al. 1987), erythrocytes from little skate (Leite and Goldstein, 1987; Goldstein and Brill, 1991), cultured astrocytes from cerebral cortex of mouse (Pasantes-Morales and Schousboe, 1988; Sánchez-Olea et al. 1991) and cultured cerebellar granule neurones from mouse (Schousboe et al. 1990, 1991) are stimulated by reduction of osmolality. In Ehrlich ascites tumour cells, however, only the efflux of taurine is stimulated, whereas influx of taurine is reduced (Hoffmann and Lambert, 1983). The properties of the influx systems for taurine that have been investigated in these cells are similar to the properties of the taurine channel in flounder erythrocytes. The susceptibility towards DIDS was another property that the taurine channel in flounder erythrocytes (Fig. 8) had in common with other transport systems for taurine that are stimulated by reduced osmolality (Goldstein et al. 1990; García et al. 1991; Garcia-Romeu et al. 1991; Goldstein and Brill, 1991; Morán et al. 1991; Sánchez-Olea et al. 1991; Schousboe et al. 1991; Kirk et al. 1992). In contrast, the effects of quinine (and the stereoisomer quinidine), furosemide and bumetanide on taurine transporters in other types of cells differ to some extent from the effects on the taurine channel in flounder erythrocytes (Fig. 8). Quinine (and quinidine) inhibit the volume regulatory efflux of taurine from rat retina (Morán et al. 1991), but these compounds have no effect on transport activity in erythrocytes from rainbow trout (Garcia-Romeu et al. 1991) and rabbit lymphocytes (García et al. 1991). Furosemide and bumetanide had a small, but significant, inhibitory effect on the transport of taurine in the flounder erythrocytes (Fig. 8). Furosemide also inhibits the volume regulatory efflux of taurine from erythrocytes from rainbow trout (Garcia-Romeu et al. 1991) and starry flounder (Kirk et al. 1992). The transport of taurine in rabbit lymphocytes, however, is inhibited by neither furosemide nor bumetanide (García et al. 1991).

The differences between the inhibitory effects of respectively quinine (and quinidine), bumetanide and furosemide on these transport systems for taurine may be due to structural differences between the various transporters. However, we cannot exclude the possibility that these apparent discrepancies are due to the experimental conditions (i.e. the concentrations of the inhibitors tested).
We presume that the differences between the effects of each inhibitor discussed above are due to the experimental conditions. Thus, the properties of the taurine channel in flounder erythrocytes are very similar to the properties of the various transport systems for taurine which have been published and referred to in the last two paragraphs. Therefore, it is tempting to suggest that, as in flounder erythrocytes, the volume regulatory efflux of taurine from these various types of cells is mediated by taurine channels independent of Na⁺. The only exception seems to be the transport pathway for taurine in Ehrlich ascites tumour cells (Lambert, 1984).

We do not know the mechanisms behind the inhibitory action of bumetanide, DIDS, furosemide and quinine on the taurine channel (Fig. 8). The facts that the addition of quinine promptly inhibited the efflux of taurine and that transport activity rapidly increased after removal of the inhibitor (Fig. 7) suggest that quinine interacted with sites on the outward-facing part of the cell membrane. After the removal of quinine, a secondary vigorous activation of the volume regulatory efflux of taurine was observed. As yet, we cannot explain this phenomenon.

In the light of the inhibitory effect of DIDS on the volume regulatory efflux of taurine from erythrocytes from little skate, Goldstein et al. (1990) suggested that this efflux is mediated by the band 3 anion antiporter. They also suggested that it is the anionic form of taurine that is released. Kirk et al. (1992), however, suggested that the osmolality-sensitive taurine influx in erythrocytes from starry flounder is mediated by anion channels. Not only taurine, but also monosaccharide and nucleoside osmo-effectors, are transported by the channels. However, DIDS also inhibits the volume regulatory efflux of K⁺ from erythrocytes from little skate (Dickman and Goldstein, 1990) and rainbow trout (Garcia-Romeu et al. 1991), and from liver cells from rat (Haddad and Graf, 1989). To our knowledge, anion antiporters and anion channels are unable to transport cations. Thus, these observations may suggest that DIDS (as well as bumetanide, furosemide and quinine) is not a specific inhibitor but interacts to a variable extent with different kinds of transporters.

The mechanisms behind the activation of the taurine channels

Incubation of the erythrocytes in a Ca²⁺-free medium with added EGTA inhibited the volume regulatory efflux of taurine (Fig. 11A). This suggests that reduction of osmolality induces influx of Ca²⁺ and increases intracellular Ca²⁺ concentration. These changes may contribute to the regulation of the taurine channel. In all types of cells investigated, it has been observed that: (1) reduction of osmolality is associated with increased influx of Ca²⁺ and/or increased cytosolic concentration of Ca²⁺ and/or (2) manipulation of the extracellular and/or intracellular concentration of Ca²⁺ affects the volume regulatory response of the cells (see Pierce and Politis, 1990).

Most effects of intracellular Ca²⁺ on cellular processes are mediated by specific Ca²⁺-binding proteins. One of these proteins is calmodulin. The anti-calmodulin drug trifluoperazine (Weiss et al. 1980) also inhibited the volume regulatory efflux of taurine (Fig. 11B). This suggests that calmodulin takes part in the activation of the taurine channel. A similar effect of trifluoperazine and other anti-calmodulin drugs has been demonstrated in erythrocytes from a bivalve mollusc (Pierce et al. 1989) and Ehrlich
ascites tumour cells (at isosmotic conditions) (Lambert, 1985). These drugs have no inhibitory effect, however, on the volume regulatory efflux of taurine from erythrocytes from little skate (Leite and Goldstein, 1987) or rabbit lymphocytes (García et al. 1991).

Thus, the results shown in Fig. 11A,B suggest that the activity of the taurine channel is controlled by Ca\textsuperscript{2+}/calmodulin. However, we cannot exclude the possibility that the effect which we registered is due to indirect effects from these substances. It has been shown that trifluoperazine has membrane effects that do not involve calmodulin. For instance, this drug blocks the influx of Ca\textsuperscript{2+} through voltage-sensitive channels in tumour cells from the anterior pituitary (Sand et al. 1983). It is also known that alterations of the extracellular concentration of Ca\textsuperscript{2+} affect the cell membrane and various transport processes, especially those that are voltage-dependent. However, at normal osmolality the removal of extracellular Ca\textsuperscript{2+} had no effect on the efflux of taurine from the flounder erythrocytes (Fig. 11A).

Metabolites of arachidonic acid also seem to contribute to the activation of the taurine channel under hyposmotic conditions. Fig. 11C shows that nordihydroguaiaretic acid completely inhibited the volume regulatory efflux of taurine. Nordihydroguaiaretic acid inhibits the enzyme 5-lipoxygenase (Cashman, 1985). This enzyme converts arachidonic acid to leukotriene-A\textsubscript{4} (LTA\textsubscript{4}). LTA\textsubscript{4} may be hydrolyzed to LTB\textsubscript{4} or conjugated with the tripeptide glutathione to produce LTC\textsubscript{4}. LTC\textsubscript{4} may then be converted to LTD\textsubscript{4} and subsequently to LTE\textsubscript{4}. As yet, we do not know which of these leukotrienes activate the taurine channel. In Ehrlich ascites tumour cells, the production of leukotrienes is stimulated by a reduction of osmolality, but only one of the leukotrienes (LTD\textsubscript{4}) accelerates the volume regulatory process (Lambert et al. 1987). The volume regulatory efflux of taurine, which is a part of this regulatory process (Hoffmann and Lambert, 1983), is also stimulated by LTD\textsubscript{4} (Lambert and Hoffmann, 1991). The volume regulatory response of rat liver cells does not seem to be controlled by leukotrienes. The response is not inhibited by nordihydroguaiaretic acid (Von Dahl et al. 1991).

The results presented in this study suggest that the volume regulatory efflux of taurine from flounder erythrocytes is mediated by a taurine channel. Ca\textsuperscript{2+}/calmodulin and leukotrienes seem to contribute to the activation of the channel. As yet, we do not know whether other cellular processes are involved.

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References
Osmolality-sensitive taurine channels


