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Comparison of the Osmolyte Transport Properties Induced by trAE1 versus $I_{Clswell}$ in *Xenopus* Oocytes

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Abstract. During cell swelling, cells release organic osmolytes via a volume-activated channel as part of the regulatory volume decrease. The erythrocyte membrane protein AE1 (band 3), has been shown to be involved in regulatory volume responses of fish erythrocytes. Previous studies showed that the expression of trout AE1 in *Xenopus laevis* oocytes induces band 3 anion exchange activity and organic osmolyte channel activity. However, an endogenous swelling-activated anion channel, $I_{Clswell}$, is present in *Xenopus* oocyte membranes. Therefore, it is not yet known whether a new organic osmolyte channel is formed or whether the endogenous channel, $I_{Clswell}$, is activated when trout AE1 is expressed in the oocytes. The purpose of this study was to determine whether the expression of trout AE1 in *Xenopus* oocytes leads to the formation and membrane insertion of a new organic osmolyte channel or activates $I_{Clswell}$. To differentiate between the two possibilities, we compared the time courses, pH profiles and inhibitor sensitivities of both trout AE1 and $I_{Clswell}$. The results of taurine-uptake experiments show that the time courses and pH levels for optimum expression of trout AE1 and $I_{Clswell}$ differ significantly. The inhibitor sensitivities of the organic osmolyte channel mediated by trout AE1 and $I_{Clswell}$ are also significantly different, strongly suggesting that the expression of trout AE1 in *Xenopus* oocytes does not activate $I_{Clswell}$, but rather forms a new organic osmolyte channel.

Key words: Organic osmolyte — Taurine — Channel — Anion exchanger — Oocyte

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Introduction

Cell volume regulation is a fundamental property of most cells and is critical for many cellular functions and processes. Upon exposure to anisosmotic media, cells initially swell or shrink, activating cellular mechanisms to restore cell volume by increasing the transport of osmotically active particles and entrained water, a process called regulatory volume decrease (RVD) or regulatory volume increase (RVI), respectively [3, 17, 20]. Cell volume regulation and the transport systems involved have been studied extensively in fish red blood cells as many of these organisms face changing osmotic environments. Thus, it is critical that their cells have the ability to regulate cell volume [7, 8]. Intracellular inorganic electyrolytes (K^+, Na^+, Cl^-) and organic osmolytes (amino acids, polyols, betaines) make up the bulk of the osmotically active particles involved in cell volume regulation but taurine accounts for up to 50% of the solutes transported during volume regulatory responses [10, 11, 18]. Previous studies have shown that in fish red blood cells, volume regulation involves a volume-activated, sodium-independent, bidirectional organic osmolyte channel with a substrate specificity that varies with different species [4, 9, 14, 16].

The volume-activated channel in trout and skate red blood cells is blocked by anion exchange (AE) inhibitors, suggesting that the red blood cell membrane protein AE1 (band 3) is involved in the volume regulatory response [7, 8, 10], a suggestion that is supported by comparative physiological studies in skates and agnathan fishes [2]. The AE1-mediated volume-activated taurine transport has been studied extensively in trout red blood cells. However, as with other putative swelling-activated channels [13, 19, 21], it has proven difficult to determine whether trout AE1 (trAE1) acts as the channel itself or whether

trAE1 activates another membrane protein to act as the channel. Trout AE1 has been cloned and expressed in the *Xenopus laevis* oocyte expression system and studies have shown that expression of trAE1 in the oocytes induces an anion conductance and taurine transport in these cells [6]. The characteristics of this transporter are similar to those found in trout erythrocytes exhibiting anion exchange and volume-activated channel activities which are sensitive to AE inhibitors [6, 17]. The channel is activated only under hypotonic conditions in trout erythrocytes but is active under isotonic conditions when expressed in *Xenopus* oocytes, suggesting that trAE1 may adopt a conformation in the oocytes that only occurs in trout red blood cells under hypotonic stress [6, 17].

An endogenous volume-activated organic osmolyte anion channel, $I_{Clswell}$, is present in the *Xenopus* oocyte membrane and has been shown to mediate taurine transport in *Xenopus* oocytes [13, 19, 20, 21]. Thus, the expression of trAE1 in oocytes may activate $I_{Clswell}$, even under isotonic conditions. The purpose of the present study was to determine whether the osmolyte channel formed when trAE1 is expressed in *Xenopus laevis* oocytes is due to trAE1 functioning as the channel per se, or is due to the activation of $I_{Clswell}$. Voets et al. compared the transport properties of the channel that was formed when the mammalian I_{Cln} was expressed in oocytes with the endogenous channel, $I_{Clswell}$. They studied the currents induced by I_{Cln} and $I_{Clswell}$, the pH profiles of these channels and the effects of extracellular nucleotides on the channel activities. They found that I_{Cln} and $I_{Clswell}$ exhibited different characteristics, suggesting that the two channels are separate and distinct [21]. A similar strategy was used in the present study, involving comparisons between the time courses, pH profiles and inhibitor sensitivities of the channel formed upon expression of trAE1 with those of $I_{Clswell}$. The results of these studies show that the characteristics of these two channels are different and suggest that trAE1, when expressed in oocytes, forms a new channel.

Materials and Methods

In Vitro cRNA Transcription

The trout RBC band-3 mRNA was prepared from full-length cDNA from trout erythrocytes supplied by Dr. H. Appelhans of the University of Frankfurt. The trAE1 cDNA was subcloned into pSP64poly(A). The cDNA was linearized by HindIII or EcoRI downstream of the cDNA before in vitro transcription. 2 μg of linearized DNA was transcribed for 2 hr at 37°C with a mixture of SP6 RNA polymerase, RNase-free H_2O (Promega, Madison, WI), capping agent GpppG and premixed rNTPs [rGTP, rATP, rCTP, rUTP]. RNAse-free DNAse was added and the mixture was incubated for 20 min at 37°C. The capped RNA was extracted with chloroform, isoamyl alcohol, 100% EtOH was added to the aqueous layer and the mixture was incubated at −80°C for 15 min, microcentrifuged for 5 min and the EtOH was poured off. The pellet was washed twice in 70% EtOH, air dried, then resuspended in ddH20 with RNAsin. RNA was stored at −80°C. An aliquot was analyzed by agarose-formaldehyde gel electrophoresis.

Oocyte Injection

The oocytes were removed from ice-anesthetized (0.3% 3-aminobenzoic acid ethyl ester) adult *Xenopus laevis* and maintained at 18°C in ND96 (in mM: 96.0 NaCl; 2.0 KC1; 1.8 $CaCl_2$; 1.0 $MgCl_2$; 5.0 HEPES; pH 7.5; 210 mOsm, filtered). The oocytes used to test for trAE1 activity were defolliculated by treatment with 2 mg/ml collagenase and 1.0 mg/ml trypsin inhibitor in calcium-free OR2 (oocyte Ringer, in mM: 82.5 NaCl, 2.5 KC1, 5.0 HEPES, 1.0 $MgCl_2$) for 45 min. The collagenase and trypsin inhibitor were aspirated off and the oocytes were washed in calcium-free OR2 three times and incubated in calcium-free OR2 for 1 hr. Then stage V–VI oocytes were selected and maintained in ND96 supplemented with penicillin (10 U/ml) and streptomycin (10 μg/ml) and sodium pyruvate (2.5 mM) at 18°C overnight and then injected with trAE1 cRNA (50 nl of 70 ng/μl). The control group consisted of oocytes defolliculated, selected and maintained as mentioned above, that were injected with 50 nl water.

The oocytes used to test for $I_{Clswell}$ activity were briefly treated with 1.0 mg/ml collagenase and 1 mg/ml trypsin inhibitor in calcium-free OR2 for 20 min. The collagenase was aspirated and the oocytes were washed in calcium-free OR2 three times, incubated in calcium-free OR2 for 1 hr, and stage V–VI oocytes were manually defolliculated. The undamaged oocytes that were successfully defolliculated were allowed to recover for 2–3 hr then injected with 50 nl of water. The oocytes were maintained at 18°C in ND96 supplemented with penicillin (10 U/ml) and streptomycin (10 μg/ml) and sodium pyruvate (2.5 mM). Control oocytes for $I_{Clswell}$ experiments were defolliculated as $I_{Clswell}$ oocytes, injected with water and tested in isotonic medium.

Radioactive Flux Measurements

For taurine-uptake experiments, $I_{Clswell}$ activity was tested 1 day post-injection and trAE1 activity was tested 3 days post-injection. 6–10 oocytes were first washed in ice-cold ND96 and then transferred to wells containing 0.4 ml ND96, 1 mM taurine and ^{3}H-taurine with a specific activity of 40,000 cpm/nmol taurine. For $I_{Clswell}$, ND96 was replaced with ND48 (in mM: 48.0 NaCl; 2.0 KC1; 1.8 $CaCl_2$; 1.0 $MgCl_2$; 5.0 HEPES; pH 8.5, filtered). Control groups were water-injected oocytes incubated in ND96, 1.0 mM taurine and ^{3}H-taurine. After incubating for a designated time, the oocytes were washed twice in ice-cold media and quickly transferred to scintillation vials. Excess extracellular fluid was quickly aspirated and 20 μl of 20% SDS were added to all the tubes. 10 μl of the incubation medium from each group was counted in duplicates. Liquid scintillation fluid was added to scintillation vials, vortexed, then placed in a liquid scintillation spectrometer and analyzed for ^{3}H-taurine. Results are presented as pmol ^{3}H-taurine/hr/oocyte.

Measurement of ^{36}Cl exchange was performed in trAE1-injected oocytes. The trAE1 oocytes were quickly washed in ice-cold Cl-free medium, then placed in wells with incubation medium: 147 μl ND96 containing ^{36}Cl with a specific activity of 350 cpm/nmol chloride and 10 μM bumetanide (to inhibit $Na^+/K^+/Cl^-$ channels). After 30-min incubation, the oocytes were removed from the wells, washed in ice-cold Cl-free media and transferred to scintillation vials. The excess extracellular fluid was quickly removed and 20 μl of 20% SDS were added to all tubes. The tubes were then vortexed and placed in a liquid scintillation analyzer to determine ^{36}Cl activity for each oocyte. Results are presented as nmol ^{36}Cl/hr/oocyte.

Pharmacological And General Inhibitors

To determine the inhibitor sensitivities of $I_{Clswell}$ and trAE1, the procedure was followed as described above in the presence of one of the following inhibitors/blockers (in mM): 0.1 DNDS (4,4′-dinitrostilbene-2,2′-disulfonic acid), 0.5 quinine, 0.1 niflumic acid, 1.0 lanthanum chloride or 5.0 cAMP. Ten-times stock solutions were made in either ND96 (for trAE1) or ND48 (for $I_{Clswell}$) for each of the inhibitors and addition of the stock solution to the incubation medium diluted the inhibitors to the desired concentrations. For lanthanum chloride experiments, oocytes were preincubated in 1.0 mM lanthanum chloride for 20 min, then the experiment proceeded as described above.

Membrane Protein Preparation And Western Blotting

A method similar to that described by Schmieder et al. was used to determine the cellular localization of trAE1 in injected oocytes [19]. Oocytes, either injected with water or trout AE1 mRNA were incubated for 72 hrs in ND96 medium as before. Fifteen oocytes were combined and homogenized in 5ml lysis buffer (50 mM Tris, pH 7.4, 66 mM ethylenediamine tetraacetic acid, 1% vol/vol Triton X-100, 0.4% wt/vol deoxycholic acid, with the Complete protease inhibitor cocktail (Roche Molecular, Indianapolis, IN). Samples were homogenized by thirty strokes in a tight-fitting Teflon pestle homogenizer and centrifuged at 1000 × g for 5 min at 4°C. The pellets were brought up in 500 μl lysis solution, homogenized again, and centrifuged at 1000 × g for 5 min at 4°C. This process was repeated a third time to clear the supernatant. The pellet was then homogenized in 500 μl lysis buffer and centrifuged at 70,000 × g for 30 min at 4°C to obtain the microsomal pellet. The pellet was resuspended in lysis buffer, the protein concentration measured by the bicinchoninic-acid procedure, and 20 μg protein was loaded on 10% SDS-PAGE. Samples were resolved and immediately transferred to a polyvinylene difluoride membrane. Blots were blocked in 5% Blotto (5% wt/vol nonfat dry milk in T-TBS (10 mM Tris pH 7.4, 140 mM NaCl, 5 mM KC1 with 0.5% vol/vol Tween 20)) and incubated overnight with either rabbit polyclonal anti-trout AE1 or murine monoclonal anti-Na^+/K^+-ATPase alpha subunit. Blots were washed five times in T-TBS, incubated with appropriate peroxidase conjugated secondary antibodies, washed five times with T-TBS and a last wash in TBS and developed using an enhanced chemiluminescent system (Supersignal, Pierce, Rockford, IL).

Female *Xenopus* oocytes were purchased from Nasco (Madison, WI) and maintained at room temperature in Cl-free water. Bumetanide, sodium pyruvate, inhibitors and salts were purchased from Sigma-Aldrich (St. Louis, MO), collagenase and trypsin inhibitor, from Life Technologies. $Na^{36}Cl$ was purchased from ICN Biomedicals (Costa Mesa, CA) and, H-taurine from NEN Life Science (Boston, MA).

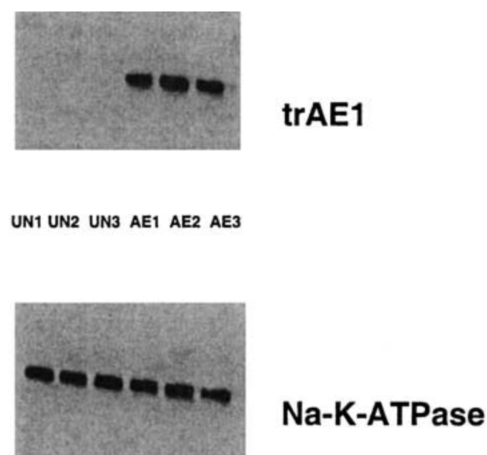

Fig. 1. Cellular localization of trout AE1 in *Xenopus* oocytes. In the top panel, a crude membrane preparation from control (lane UN1–UN3)- and trAE1-injected (AE1-AE3) oocytes were tested for immunoreactivity with polyclonal antibody raised against the N-terminus of trAE1 (97 kD). In the bottom panel, crude membrane preparations from control (UN1–UN3)- and trAE1 (AE1-AE3) injected oocytes were tested for immunoreactivity with antibody raised against the alpha subunit of the Na^+/K^+-ATPase (103 kD).

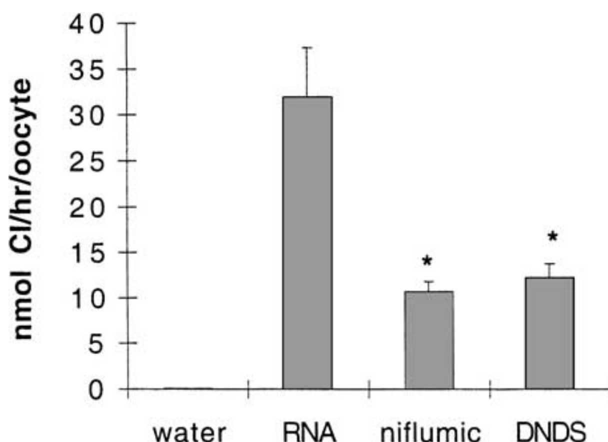

Fig. 2. Chloride exchange (uptake) across the plasma membrane in oocytes expressing trAE1, compared to water-injected control oocytes. The effects of known anion exchange inhibitors, niflumic acid (0.1 mM) and DNDS (0.1 mM), on trAE1. Values are expressed as mean ± SE (n = 10). Asterisk denotes data significantly different from uninhibited chloride exchange (p < 0.01).

Results and Discussion

Trout AE1 Cellular Localization

Trout AE1 cRNA was injected into *Xenopus* oocytes in order to determine the cellular localization of the trAE1 channel and to ensure that the exogenous cRNA was being expressed. A crude membrane preparation from water-injected and trAE1 cRNA-injected *Xenopus* oocytes were examined for immunoreactivity with polyclonal N-terminal trAE1 antibody by Western blot. Three groups of water-injected oocytes and three groups of trAE1-injected oocytes were tested (Fig. 1). In each of the membranes from oocytes injected with trAE1 cRNA, a predominant band at 97 kDa was observed, but there was no immunoreactivity when the membranes from water-injected oocytes were used. The six groups of membranes from oocytes, water- and trAE1-injected,

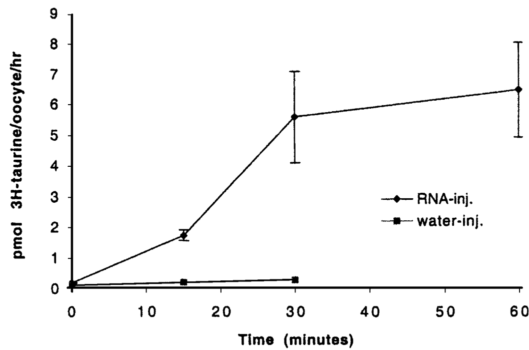

Fig. 3. Time course of taurine uptake in oocytes after trAE1 expression at 0, 15, 30 and 60 min (diamonds) and the time course of taurine uptake in water-injected oocytes at 0, 15 and 30 min (squares). Values are mean ± SE ($n = 10$).

all tested positive for the Na^+/K^+-ATPase, α subunit. These results indicate that the trAE1 cRNA is translated by the oocytes and that the resulting protein is localized in the membrane fraction.

Trout AE1 Expression in *Xenopus* Oocytes: ^{36}Chloride Exchange

In trout erythrocyte membranes, there is an anion exchanger, AE1, that exhibits rapid exchange of intracellular bicarbonate with extracellular chloride in an electroneutral anion exchange [12, 17]. Therefore, chloride exchange (uptake) experiments were performed as an additional method of demonstrating AE1 expression and insertion in the oocyte membrane. Figure 2 shows chloride uptake in trAE1 RNA- and water-injected oocytes. There is a significant increase in chloride uptake in oocytes expressing trAE1 compared to the control, water-injected oocytes, suggesting that the trAE1 RNA injection induces anion exchange activity. The anion exchanger in trout erythrocytes as in *Xenopus* oocytes is blocked by application of the pharmacological inhibitors, niflumic acid and DNDS [7]. Thus, these anion-exchange inhibitors were used to determine whether the anion-exchange activity in the trAE1-injected oocytes is also blocked in a similar manner, as was shown by Fievet et al. [6]. As illustrated in Fig. 2, niflumic acid and DNDS significantly inhibited chloride uptake by 67% and 62%, respectively. The significant increase in chloride exchange and inhibitor sensitivities observed in the trAE1-injected oocytes compared to water-injected oocytes suggest that the observed stimulation in chloride uptake was due to trAE1 activity. These results and those observed above in the Western blot experiments indicate that when injected into *Xenopus* oocytes, the exogenous trAE1 cRNA was translated and that a functional trAE1 protein was produced and inserted into the oocyte plasma membrane where it is able to mediate the anion-exchange activity.

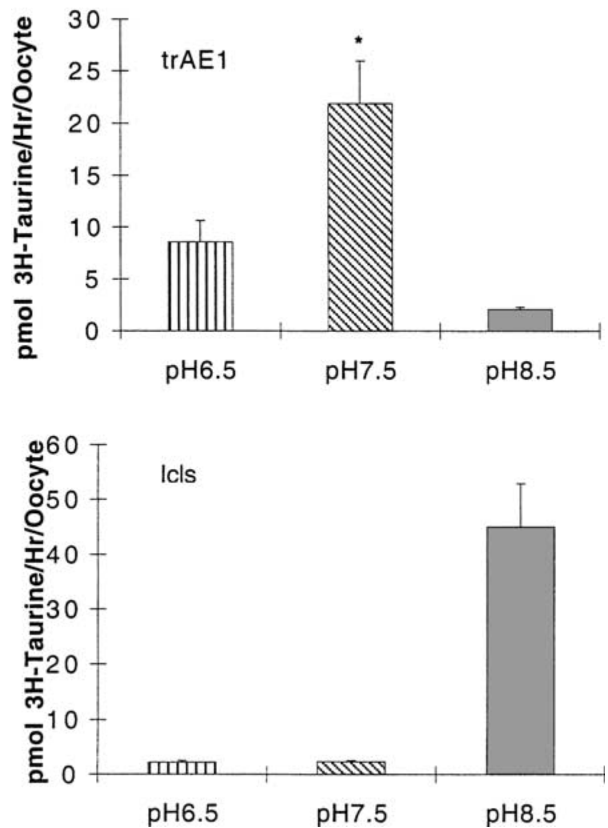

Fig. 4. pH profiles of trAE1- and $I_{Clswell}$-mediated taurine uptake performed in isotonic (ND96) and hypotonic (ND48) media, respectively, at pH 6.5, 7.5 and 8.5. Striped bars, cross hatched and solid bars represent taurine uptake at pH 6.5, 7.5 and 8.5, respectively. In this and subsequent figures, $I_{Clswell}$ was measured in water-injected oocytes. Values are mean ± SE ($n = 8$). Asterisk: data significantly different from other pH ($p < 0.01$).

Trout AE1-Mediated Taurine Uptake

In trout erythrocytes, the anion exchanger AE1 (band 3), is involved in the volume-activated organic osmolyte/anion channel activity [7, 11]. Upon volume expansion, trout erythrocytes initially swell then undergo regulatory volume decrease, transporting electrolytes and organic osmolytes across the cell membrane followed by entrained water. The β-amino acid taurine plays a significant role in regulatory volume decrease when red blood cells are swollen by hypotonic stress [11, 12, 17]. To study the involvement of trAE1 in regulatory volume responses via the volume-activated organic osmolyte channel further, the transport of taurine was investigated in trAE1 and water-injected oocytes. The *Xenopus* oocyte expression system is suitable for studying the trAE1-mediated osmolyte channel because, as previous studies have shown, collagenase-treated oocytes have a low level of endogenous channel activity [6]. Thus, control oocytes are almost impermeable to taurine.

The taurine-uptake time course was determined 48 and 72 hrs after oocytes were injected with trAE1,

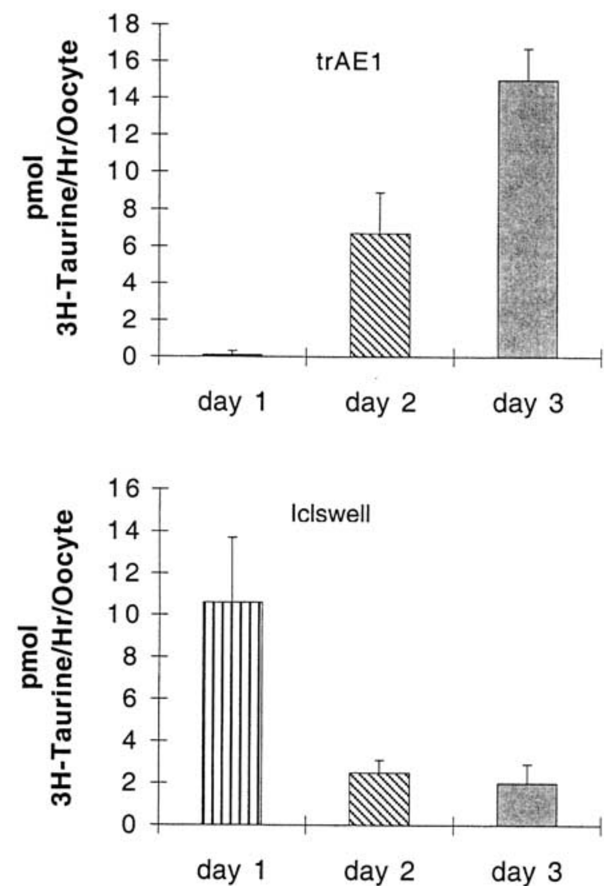

Fig. 5. Post-injection time course of trAE1-and $I_{Clswell}$-mediated taurine uptake performed in isotonic medium (ND96) and hypotonic medium (ND48), respectively, 24, 48 and 72 hrs after trAE1 cRNA or water was injected into oocytes. Striped bars, cross-hatched and solid bars represent taurine uptake 24, 48 and 72 hrs post-injection, respectively. Values are means ± SE ($n = 10$).

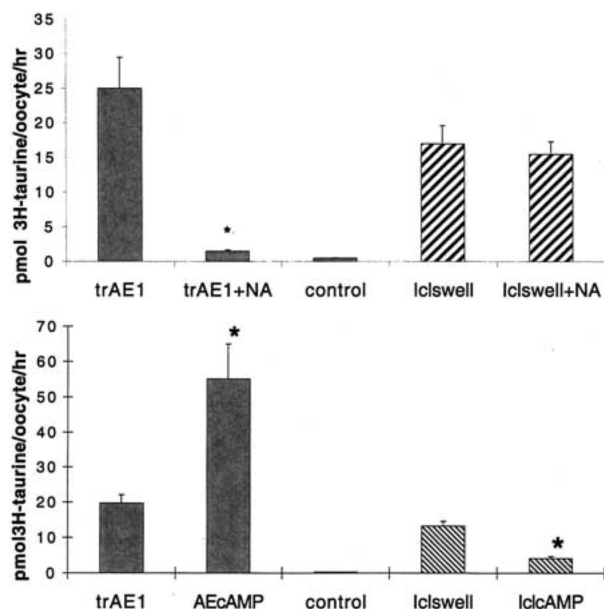

Fig. 6. Effects of known anion exchange inhibitor, 0.1 mM niflumic acid (top panel), and $I_{Clswell}$ inhibitor, 5.0 mM cAMP (bottom panel), on trAE1- and $I_{Clswell}$-mediated taurine uptake. Solid bars and cross-hatched bars represent trAE1- and $I_{Clswell}$-mediated taurine uptake, respectively. Values are mean ± SE ($n = 12$ for niflumic acid, $n = 22$ for cAMP). Asterisk: data significantly different from control condition ($p < 0.01$).

in isosmotic media. Taurine uptake in trAE1-injected oocytes 48 hr post-injection varied significantly in each batch of oocytes, but was more consistent 72 hrs post-injection (*data not shown*). Thus, subsequent experiments on trAE1-injected oocytes were performed 72 hrs post-injection. As illustrated in Fig. 3, taurine uptake increased over a 60-min period. From 0 to 15 min, there was an influx of 1.74 ± 0.18 pmol taurine, from 0 to 30 min, an influx of 5.6 ± 1.5 pmol, and from 0 to 60 min, an influx of 6.5 ± 1.6 pmol. There was a marked increase in the rate of taurine uptake from the 15-min time point to the 30-min time point. Taurine uptake was near maximal at 30 min. Therefore all subsequent experiments were performed at 30 min.

Trout AE1 and $I_{Clswell}$ Activities

In *Xenopus laevis* oocytes, hypotonic swelling has been found to activate an endogenous organic osmolyte efflux pathway. This volume-activated transport is thought to occur via $I_{Clswell}$, an endogenous volume-activated anion/organic osmolyte channel in *Xenopus* oocytes [6, 13, 19, 20, 21]. $I_{Clswell}$ has both anion conductance and organic osmolyte channel activity. Thus, the question arises as to whether the osmolyte channel activity observed following the expression of trAE1 in *Xenopus* oocytes activates $I_{Clswell}$ or results in the formation of a new osmolyte channel [6]. Therefore, to further investigate this question, a series of experiments was performed in order to compare and contrast the characteristics of $I_{Clswell}$- and trAE1-mediated taurine transport in *Xenopus* oocytes. The activities of trAE1 and $I_{Clswell}$ were measured at pH 6.5, 7.5 and 8.5. As shown in Fig. 4, the optimum pH for trAE1 and $I_{Clswell}$ activities are 7.5 and 8.5, respectively. The latter pH optimum, for $I_{Clswell}$, is similar to that found by Ackerman et al. [1]. The taurine uptake in trAE1-injected oocytes at pH 7.5, 21.9 ± 4.1 pmol taurine/oocyte/hr, is significantly higher than at 6.5 or 8.5 ($p < 0.01$), 8.6 ± 2.0 and 2.1 ± 0.2 pmol taurine/oocyte/hr, respectively. The taurine uptake via $I_{Clswell}$ at pH 8.5, 45 ± 7.9 pmol taurine/oocyte/hr, is significantly higher than at 6.5 or 7.5 ($p < 0.01$), 2.2 ± 0.3 and 2.3 ± 0.2 pmol taurine/oocyte/hr, respectively.

The post-injection time course (Fig. 5) shows different trends between the activities of trAE1 and $I_{Clswell}$ 1–3 days after injection. The activity of trAE1

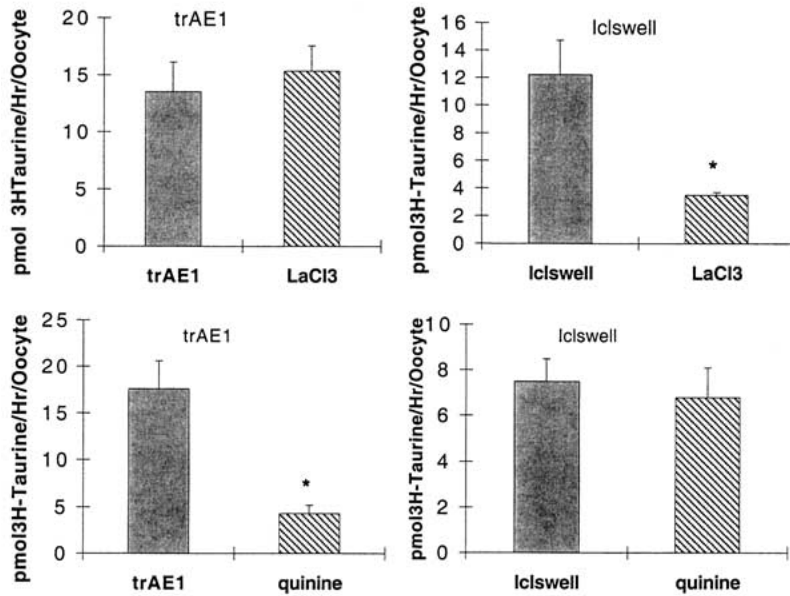

Fig. 7. Effects of 1.0 mM $LaCl_3$ (top panel) and 0.1 mM quinine (bottom panel), on trAE1- and $I_{Clswell}$-mediated taurine uptake. Solid bars represent taurine uptake and cross-hatched bars represent inhibition of taurine uptake by quinine or $LaCl_3$. Values are mean ± SE ($n = 19$ quinine, $n = 24$ $LaCl_3$). Asterisk: data significantly different from uninhibited condition ($p < 0.01$).

increases with time, as shown by the increase in taurine uptake at 1, 2 and 3 days post-injection (with trAE1 cRNA). In contrast, $I_{Clswell}$ activity decreases with each subsequent day, as shown by the decreasing levels of taurine uptake at 1, 2 and 3 days post-injection (with water).

The results of the pH profile and expression time course experiments show that the organic osmolyte transport mechanism that is induced by expression of trAE1 in *Xenopus* oocytes has characteristics different from the endogenous volume-activated organic osmolyte/anion transport via $I_{Clswell}$. Maximal activity of trAE1 is observed at pH 7.5 and increases with time, while the maximal activity of $I_{Clswell}$ is observed at pH 8.5 and decreases with time. Subsequent experiments investigating trAE1 activity were performed 3 days post-injection in isosmotic media at pH 7.5, using collagenase-defolliculated oocytes, while experiments on $I_{Clswell}$ were performed 1 day post-injection in hyposmotic media at pH 8.5, using mechanically defolliculated oocytes.

Pharmacological Blockers and Inhibitors

Davis-Amaral et al. showed that quinine inhibits the AE-mediated volume-sensitive taurine transport in skate red blood cells [4]. Previous studies have also shown that in skate as well as trout red blood cells, niflumic acid inhibits the AE-mediated volume-sensitive transport of taurine [4, 7]. Earlier studies showed that in *Xenopus* oocytes, lanthanum chloride and cAMP inhibit anion conductance and taurine transport via $I_{Clswell}$ [1, 20]. Therefore, the effects of quinine, niflumic acid, lanthanum chloride and cAMP on $I_{Clswell}$- and trAE1-mediated channel activity were investigated in *Xenopus* oocytes. The inhibitor sensitivities of trAE1 were compared to that of $I_{Clswell}$. The anion exchange blocker, niflumic acid, significantly inhibited trAE1-mediated taurine uptake by 94%, with no significant effect on taurine uptake via $I_{Clswell}$ (Fig. 6). Interestingly, cAMP stimulated trAE1 almost 2-fold, whereas it significantly inhibited $I_{Clswell}$ by 69% (Fig. 6). Quinine inhibited taurine transport via trAE1 by 76%, with no significant effect on $I_{Clswell}$ (Fig. 7). Lanthanum chloride, on the other hand, slightly stimulated (13%) the trAE1 mediated pathway but significantly inhibited $I_{Clswell}$ by 71% (Fig. 7).

In conclusion, comparing the characteristics of trAE1-mediated and $I_{Clswell}$-mediated taurine transport, we found that trAE1-mediated taurine transport was optimal when the experiments were performed on collagenase-defolliculated oocytes, 3 days post-injection, in isosmotic media at pH 7.5. This pathway was significantly inhibited by niflumic acid and quinine, not inhibited by lanthanum, and was significantly stimulated by cAMP. $I_{Clswell}$-mediated taurine transport was optimal when experiments were performed on manually defolliculated oocytes, 1 day post-injection, in hyposmotic media with pH 8.5, and this pathway was significantly inhibited by lanthanum and cAMP, but not by niflumic acid and quinine. These results suggest that trAE1 does not activate $I_{Clswell}$ but rather either forms the organic osmolyte channel itself or perhaps activates another endogenous channel.

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