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Physiology

Transport of uncharged organic solutes in *Xenopus* oocytes expressing red cell anion exchangers (AE1s)

(anion exchanger/Cl channel/taurine transport/urea transport/volume regulation)

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ABSTRACT **When expressed in *Xenopus* oocytes, the trout red cell anion exchanger tAE1, but not the mouse exchanger mAE1, elicited a transport of electroneutral solutes (sorbitol, urea) in addition to the expected anion exchange activity. Chimeras constructed from mAE1 and tAE1 allowed us to identify the tAE1 domains involved in the induction of these transports. Expression of tAE1 (but not mAE1) is known to generate an anion conductance associated with a taurine transport. The present data provide evidence that (*i*) the capacity of tAE1 and tAE1 chimeras to generate urea and sorbitol permeability also was associated with an anion conductance; (*ii*) the same inhibitors affected both the permeability of solutes and anion conductance; and (*iii*) no measurable water transport was associated with the tAE1-dependent conductance. These results support the view that fish red blood cells, to achieve cell volume regulation in response to hypotonic swelling, activate a tAE1-associated anion channel that can mediate the passive transport of taurine and electroneutral solutes.**

Hypotonically swollen cells recover their volume (regulatory volume decrease) by releasing intracellular solutes (ions and organic compounds) and obligated water. Taurine, an amino acid that is present in various cells at concentrations of up to 40 mM, is often the primary organic osmolyte involved in regulatory volume decrease. But, in some cells, polyols, such as sorbitol and myoinositol, play an important role (1). Volume-activated amino acid and polyol efflux mechanisms share two characteristics (1): (*i*) they are mediated by passive, Na-independent transport pathways having the characteristics of anion channels and (*ii*) they are similarly affected by various anion transport blockers, suggesting that transport of these structurally unrelated compounds may be mediated by a single pathway.

Fish erythrocytes respond to hypotonic swelling by inducing a loss of K^+ and Cl^- and also a transport of taurine and polyols (2–8) similarly affected by various anion transport blockers (5, 7–9). Recent observations (10) show that: (*i*) expression of the trout anion exchanger AE1 (tAE1) in *Xenopus* oocyte elicits, in addition to anion exchange activity, both anion conductance and appearance of a passive taurine transport; (*ii*) a very tight correlation exists between expression of anion conductance and taurine transport; (*iii*) compounds that inhibit anion conductance also inhibit taurine transport; (*iv*) expression of the anion exchanger (mAE1) of mouse erythrocyte, a cell that does not release organic osmolytes in response to swelling, elicits anion exchange activity but neither conductance nor taurine transport. Taken together, these results indicate that the tAE1-induced channel mediates a taurine transport and suggest that, in fish red blood cells, the volume-activated taurine transport is associated with the anion exchanger (band 3 protein).

Hyposmotic swelling of trout red blood cells induces not only taurine transport (4) but also a transport of sorbitol that is inhibited by the anion transport blockers DIDS and niflumic acid (unpublished data). The purpose of the present study was to evaluate further the transport capacities of red blood cell anion exchangers when they are expressed in oocytes. We found that expression of trout anion exchanger tAE1, but not of mouse exchanger mAE1, generated a transport of the electroneutral solutes sorbitol and urea. There is a specific erythroid urea transporter, hUT-B1 (original name HUT11) [having been cloned (11)], and a comparative analysis of hUT-B1- and tAE1-induced urea transport has been performed. Chimeras between tAE1 and mAE1 have been constructed and expressed to determine the domains of tAE1 that critically are associated with urea transport. This study demonstrates that the tAE1-induced urea permeability directly depended on the anion conductance generated by tAE1 expression. The structure–function analysis confirmed the correlation existing between urea transport and the capacity of chimeras to generate an anion conductance. The results indicate that urea, sorbitol, and taurine share the tAE1-dependent channel.

EXPERIMENTAL PROCEDURES

Production of cRNA. The following plasmids were used to transcribe cDNAs encoding for the transporters expressed in this report: pSP64poly(A)-TB3 encodes for trout erythroid band 3 (10); pSPT19-mAE1 encodes for mouse erythroid band 3; pGEM4ST-UT.B1 encodes for human bone marrow urea transporter; pGEM4ST-AQP1 encodes for frog urinary bladder aquaporin, a specific water channel; and the transcription vector pGEM4ST contains 5′ and 3′ untranslated regions of *Xenopus* β-globin for optimal oocyte expression. The chimerical constructs and deletion mutants (see *Results* for details) were inserted into pSP64polyA or pSPT19 and were obtained as described in ref. 10. All of these plasmids were linearized downstream of the cDNA before *in vitro* transcription by using SP6 or T7 RNA polymerase (Promega), and cRNA transcription was obtained as described in ref. 10.

Oocyte Injection. Oocytes were removed from ice-anesthetized *Xenopus* and maintained at 18°C in modified Barth's saline (MBS composition in mM: NaCl 85; KCl 1; $NaHCO_3$ 2.4; $MgSO_4$ 0.82; $Ca(NO_3)_2$ 0.33; $CaCl_2$ 0.41; Hepes 10; and NaOH 4.5 pH 7.4 and supplemented with penicillin 10 units/ml and streptomycin 10 μg/ml). After washing with

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Abbreviation: MBS, modified Barth's saline.

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MBS, defolliculation was obtained by 16 h of incubation at 18°C in MBS containing 1.3 mg/ml collagenase (Serva) followed by a 30-min incubation in Ca-free MBS. Stage V–VI oocytes then were injected with 50 nl of 70 ng/μl cRNA [3.5 ng/oocyte, ensuring saturating expression level (10)] and were maintained at 18°C. Expression was tested 1 day postinjection and followed for up to 6 days.

Electrophysiology. Electrophysiological parameters were measured at room temperature as described (10) by using the two-electrode voltage clamp technique with a TEV 200 amplifier (Dagan Instruments, Minneapolis) monitored by computer through Digidata 1200 A/D converter/PC clamp software (Axon Instruments, Foster City, CA).

Radioactive Flux Measurements. For chloride influx measurements, eight oocytes were incubated at 18°C in 100 μl of MBS containing ^{36}Cl (Amersham) with a specific activity of 350 dpm/nmol chloride. After 15 min of incubation, the oocytes were washed twice in ice-cold MBS and transferred individually into counting vials. Excess extracellular fluid was removed quickly, and 20 μl of 20% SDS was added before vortexing. Radioactive chloride uptake in each oocyte was determined after scintillation counting with an external standard procedure to correct for quenching. The incubation medium was counted in duplicate on 5-μl aliquots by using the same protocol to determine the specific activity in each experiment. Chloride uptake was calculated as the mean of the eight values and was expressed as pmol/min·oocyte. Urea influx measurements were carried out as chloride influx in MBS supplemented with 0.5 mM urea (Merck) and containing ^{14}C-urea (Amersham) with a constant specific activity of $8{\cdot}10^3$ dpm/nmol urea. Oocytes were preincubated for 1 h before ^{14}C-urea flux measurements with the urea transport inhibitors 1-(2-monochlorophenyl)-2-thiourea (MCPTU) and para-chloromercuribenzene sulfonate (pCMBS). Oocytes were not preincubated with other inhibitors: phloretin, 5-nitro-2(3-phenylpropylamino) benzoic acid (NPPB), niflumic, acid and flufenamic acid.

Water Permeability Measurements. Osmotic water permeability of oocytes was measured by using a swelling assay as previously described (12). The oocytes, maintained in MBS (200 milliosmol/kg H_2O) at 18°C, were transferred to distilled water while being viewed by transmitted light on a phase-contrast microscope connected to an image-processing system. Assuming the oocyte is spherical, the water permeability was calculated from the initial oocyte volume V_0, the initial slope of the time-course of V/V_0 [$d(V/V_0)/dt$], the initial surface S_0, and the molar volume of water V_w (= 18 cm^3/mol) with the following relationship:

$$P_f = V_0\,[d(V/V_0)/dt]/[S_0{\cdot}V_w{\cdot}(Osm_{in} - Osm_{out})],\ \text{in cm/s.}$$

RESULTS

Urea Transport by *Xenopus* Oocytes Expressing AE1s. As demonstrated (10), when expressed in oocytes, both trout (tAE1) and mouse (mAE1) anion exchangers elicited chloride exchange activity (Fig. 1*A*, open bars). But, as shown in Fig. 1*B*, expression of tAE1 also generated urea transport; control water-injected oocytes exhibited a very slow and linear uptake whereas the amount of urea taken up by tAE1-injected oocytes was much higher and similar to that accumulated into oocytes expressing the human erythroid urea transporter hUT-B1. As illustrated in Fig. 1*A*, tAE1 and hUT-B1 caused a 16- to 19-fold increase in the oocyte plasma membrane urea permeability. On the other hand, although mAE1 and tAE1 induced a similar chloride flux, mAE1 did not increase the urea flux across oocytes. Thus, not all isoforms of the AE1 family can generate urea transport when expressed in oocytes.

Urea uptake was measured in the presence of known red cell urea transport inhibitors (phloretin and pCMBS) or in the presence of urea analogues (thiourea, MCPTU). As shown in Fig. 2*A*, both types of compounds inhibited quite similarly tAE1- and hUT-B1-induced urea transport. Conversely in the presence of known AE1 inhibitors (niflumic acid, flufenamic acid, NPPB), urea transport remained unaffected in oocytes expressing hUT-B1 but was inhibited strongly in oocytes expressing tAE1 (Fig. 2*B*). It is noteworthy that DIDS, which is a classical inhibitor of anion exchanges in red cells, including trout red cells, does not affect Cl^- exchange mediated by tAE1 when this exchanger is expressed in oocytes (10). DIDS also did not affect tAE1-induced urea transport (not shown). Thus, the transport of urea generated by expression of tAE1 in oocytes was blocked specifically by compounds that inhibit the tAE1-mediated chloride flux, suggesting that tAE1 catalyzes urea movement.

Next, we analyzed the effects of anion exchangers/hUT-B1 coexpression on urea permeability. The mouse anion exchanger mAE1 elicited chloride flux and thus was expressed functionally when the cRNA was coinjected with hUT-B1 (data not shown). However, Fig. 3*a* shows that coexpression of hUT-B1 and mAE1 gave the same signal for urea transport as hUT-B1 alone, indicating no influence of mAE1 on hUT-B1 activity. Coinjection of hUT-B1- and tAE1-cRNA elicited a signal corresponding to the addition of the two individual signals (Fig. 3*b*). Moreover, in the presence of the AE1 inhibitor niflumic acid, the signal returned back to the signal of hUT-B1 alone (Fig. 3*b*). Thus, urea transport by hUT-B1 and tAE1 are additive and independent, indicating that tAE1, as mAE1, did not interact with the urea transporter under our experimental conditions.

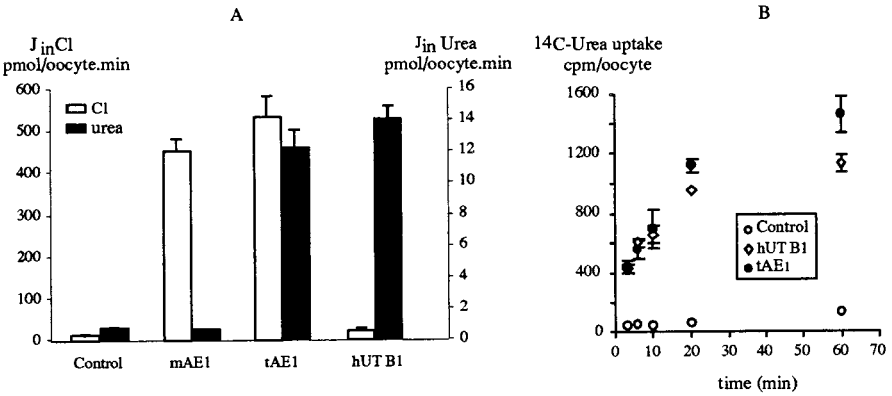

FIG. 1. Chloride and urea transport across the oocyte membrane after expression of the erythroid urea transporter hUT-B1 or red blood cells anion exchangers (tAE1, trout exchanger; mAE1, mouse exchanger). (*A*) Chloride exchange and urea transport of mAE1, tAE1, and hUT-B1 expressed in oocytes (compare with water-injected control). The flux was calculated from initial rates of uptake (5 min ^{14}C-urea uptake; 15 min ^{36}Cl uptake) after 5 days postinjection (incubation at 18°C). (Bars = mean ± SE; number of oocytes, n = 8.) (*B*) Time course of urea uptake in oocyte expressing tAE1 and hUT-B1 (compare with water-injected control). (n = 8.)

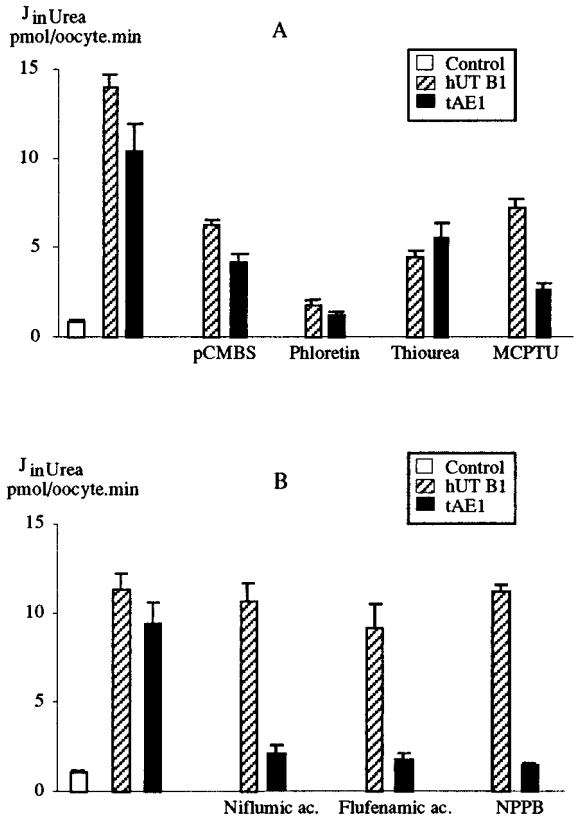

FIG. 2. Inhibition of hUT-B1-mediated and tAE1-induced urea transport by (*A*) known red blood cell urea transport inhibitors (phloretin 1 mM, pCMBS 1 mM) and urea analogues (thiourea 40 mM, MCPTU 1 mM). (*B*) AE1 inhibitors (niflumic acid, flufenamic acid, and NPPB; $5{\cdot}10^{-4}$ M). ($n = 8$.)

Relationship Among Urea Transport, Chloride Exchange, and Anion Conductance. Expression of tAE1 in *Xenopus* oocytes increases between days 1 and 6 post-cRNA injection (10). By performing measurements at different days postinjection and by injecting various nonsaturating concentrations of cRNA, we obtained a wide range of tAE1 expression levels quantified by Cl fluxes. Fig. 4*A* shows the relationship between tAE1-induced urea transport and tAE1-mediated chloride flux; chloride exchange and urea transport were correlated (closed circles). At low levels of tAE1 expression ($0 < \text{JinCl} < 100$ pmol/min), however, urea transport was not significantly increased, suggesting a nonstrict linear relationship between chloride flux and urea transport. It has been shown (10) that tAE1 expressed in oocyte generates an anion conductance and that a sigmoidal relationship exists between anion conductance and chloride exchange. We have then plotted tAE1-induced urea transport vs. tAE1-induced anion conductance (Fig. 4*B*). Urea transport appeared more simply related to anion conductance than to chloride exchange, particularly at low levels of tAE1 expression; urea transport increased as a function of anion conductance. Fig. 4*A* (open squares) shows, by contrast, that the mouse anion exchanger mAE1 did not generate urea transport whatever its level of expression. It must be noted that mAE1 also does not generate anion conductance whatever its level of expression (10). In summary, the anion exchanger that does not generate an anion conductance across the oocyte membrane (mAE1) also did not induce an urea transport. In contrast, the anion exchanger generating a conductance (tAE1) induced an urea transport, and the greater the anion conductance, the greater the urea transport. It must be pointed out that the same inhibitors affecting both anion conductance and chloride exchange (10) also affect urea transport (Fig. 2*B*).

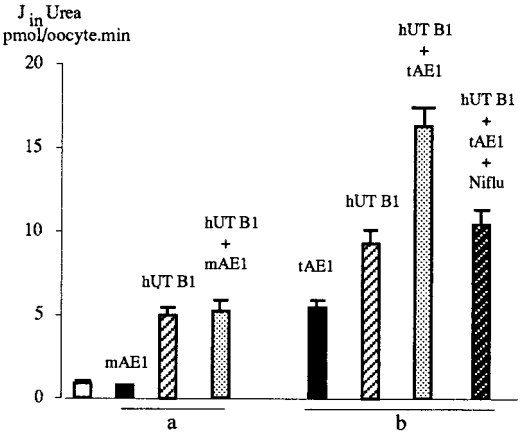

FIG. 3. Effect of coexpression of hUT-B1 and AE1s on urea transport. (*a*) Urea uptake mediated by hUT-B1 was not altered by coexpression of mAE1. (*b*) Urea uptake in oocytes expressing both hUT-B1 and tAE1 was equivalent to the addition of urea uptake induced separately by each transporter. Added to oocytes expressing both hUT-B1 and tAE1, niflumic acid blocked a part of urea uptake quantitatively equivalent to the tAE1-induced urea uptake. ($n = 8$.) In these coexpression experiments, a nonsaturating concentration of cRNA (1.7 ng) was injected and 3-min urea uptakes were measured to ensure measurements of reliable initial rates.

AE1-Structure and Urea Transport Capacity. Thus, when expressed in oocytes, both tAE1 and mAE1 exchange chloride but only tAE1 generates urea transport. To try to define the structural domain(s) involved in urea transport we expressed chimeras previously constructed (10) from these two homologous proteins and some deletion mutants (Fig. 5*A*). A significant difference in the primary structures of tAE1 and mAE1 is the extracellular loop between the putative transmembrane helices 5 and 6, which is twice as long in tAE1 (referred to as Z-loop, 50 aa acids) as in mAE1 (referred to as m-loop, 25 aa). We have swapped these extracellular loops between the mouse and the trout isoforms; tAE1 with the m-loop is referred to as TmT and the symetrical construct, mAE1 with the Z-loop, is referred to as MZM. We also have constructed chimeras in which the domains from the putative transmembrane helice 6 up to the C terminus of the protein were swapped between the trout and the mouse isoforms. The trout anion exchanger with the mouse C-terminal domain is referred to as TZM, and the mouse anion exchanger with the trout C-terminal domain is referred to as MmT. In addition a first mutant, termed "tAE1 Z(−)," was constructed from tAE1; the length of the Z-loop has been reduced to that of its mouse counterpart by deletion of 24 aa. In a second one, "termed tAE1 ΔN," the 311 N-terminal residues have been deleted. The cDNAs encoding for all of these proteins were transcribed and expressed in oocytes, and Cl and urea transport capacities were determined. Fig. 5*B* shows the relationship between chloride exchange and urea transport in oocytes expressing these different AE1 constructs as well as the average relationship previously shown in Fig. 4*A* with the wild types tAE1 and mAE1 (represented by dotted and dashed lines, respectively; experimental points omitted for clarity). The following messages emerge from comparing the relationship for the AE1 constructs with that for the wild types. [nlist]

The hydrophilic N terminus of AE1 is a domain that links the band 3 protein to the cytoskeleton. Deletion of this domain did not affect the relationship between Cl exchange and urea transport, i.e., tAE1 ΔN (Fig. 4*A*, closed squares) behaved as the wild-type tAE1. It is noteworthy that such a deletion also does not affect the anion conductance normally induced by tAE1 (10).

When the C-terminal domain of tAE1 was replaced by its mouse counterpart (TZM, Fig. 4*A*, open circles), the expres-

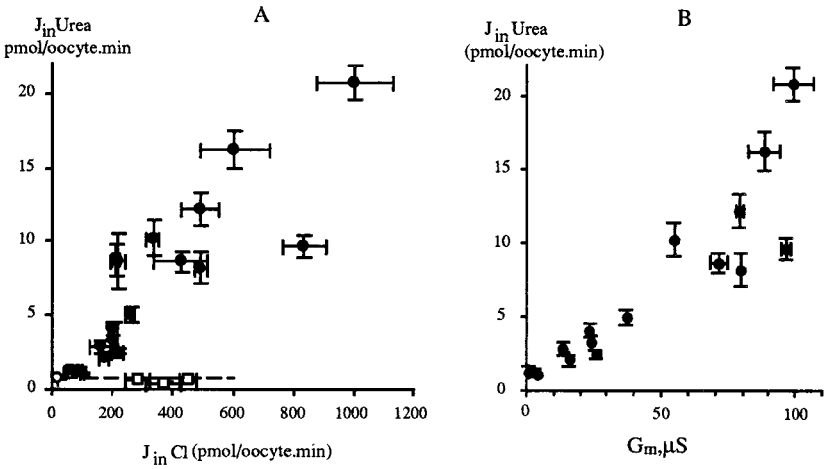

FIG. 4. Correlation between urea flux, chloride flux and anion conductance. (*A*) Urea flux vs. chloride flux for oocytes expressing tAE1 (closed circles) and mAE1 (open squares). (mean ± SE; $n = 8$ for J_{in}Cl and J_{in}Urea. (*B*) Urea flux vs. anion conductance, Gm, for oocytes expressing tAE1. Anion conductance was measured by the two electrode voltage clamp technique (see *Experimental Procedures*). ($n = 8$.)

sion pattern changed dramatically; the protein now behaved as mAE1, preserving Cl exchange activity but losing urea transport capacity. It is noteworthy that TZM also fully loses the capacity to generate an anion conductance (10).

Replacement of the trout Z-loop by its shorter mouse counterpart, TmT, has been shown to maintain chloride exchange but to reduce drastically both anion conductance and taurine transport (10). Unfortunately, for unknown reasons, it has been impossible in the present set of experiments to obtain a sufficient level of TmT expression (Fig. 4*A*, closed triangles) that would allow an accurate determination of the effect of Z-loop replacement on urea transport. Shortening of the

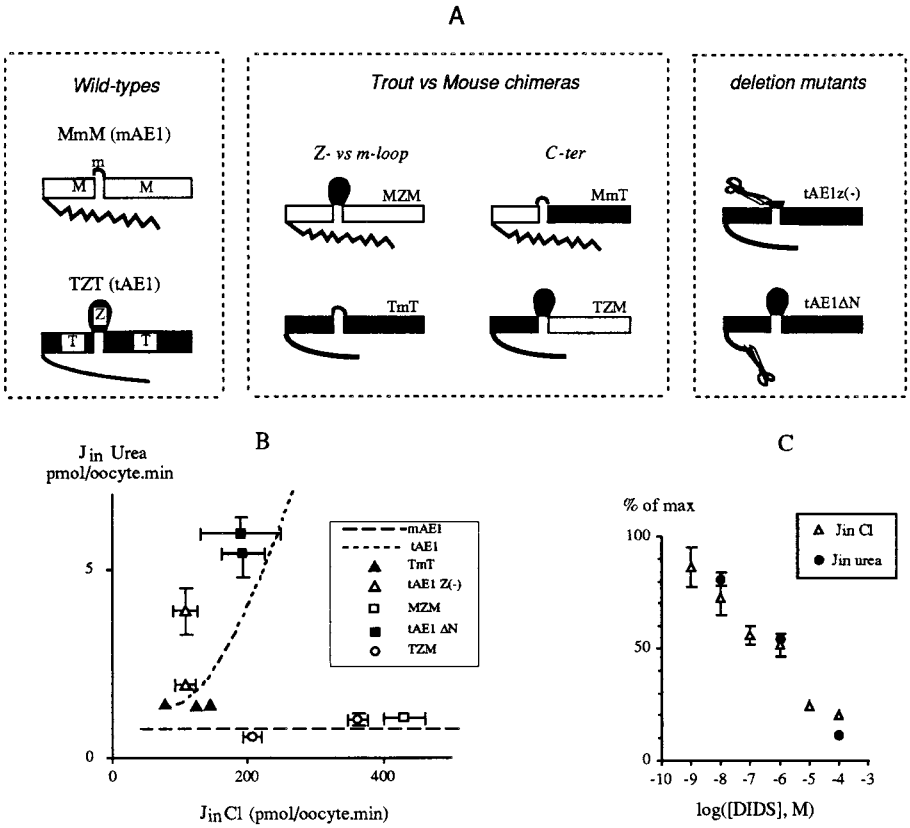

FIG. 5. Permeabilities of chimeras and deletion mutants. (*A*) Construction of chimeras and deletion mutants. The extracellular loops between transmembrane helices 5 and 6 are termed "Z" and "m" for the trout and mouse exchangers, respectively. The domains of proteins up and down the major extracellular loops are termed "T" and "M" for the trout and mouse, respectively. Thus, wild-type proteins mAE1 and tAE1 are termed "MmM" and "TZT," respectively. Trout vs. mouse chimeras: MZM, TmT, TZM, MmT (this last chimera, MmT, was not expressed functionally). Deletion mutants: tAE1 z(−) is devoid of amino acids 551–574 located in the extracellular loop between the putative transmembrane helices 5 and 6 of the trout exchanger; tAE1 ΔN is devoid of amino acids 1–311. (*B*) Correlation between urea permeability and chloride permeability for oocytes expressing several chimeras (TmT, TZM, MZM) or tAE1 mutants [tAE1 $Z^{(-)}$, tAE1 ΔN]. Values are means from eight individual measurements (sometimes error bars are hidden by symbols). The average equivalent relationships established for tAE1 and mAE1 (see Fig. 4*A*) are indicated by dotted and dashed lines, respectively, the experimental points being omitted for the purpose of clarity. (*C*) Inhibitory effect of DIDS on both chloride flux and urea transport in TmT-expressing oocytes. ($n = 8$.)

Z-loop [tAE1 Z(−), Fig. 4*A*, open triangles] did not affect urea transport. It also does not affect anion conductance (10). Introduction of Z-loop into mAE1 (MZM, Fig. 4*A*, open squares) did not induce urea transport. It also does not induce anion conductance, MZM behaving as mAE1 (10). In summary, the structure–function analysis pointed again to a close relationship between urea transport and anion conductance.

We point out above that DIDS does not affect chloride exchange mediated by tAE1 when the exchanger is expressed in oocytes. Curiously, however, DIDS inhibits Cl^- exchange in oocytes expressing the chimeric construct TmT (10). We tested then the capacity of DIDS to affect the small TmT-induced urea transport. As shown in Fig. 5*C*, DIDS inhibited this transport, and the dose dependence for inhibition of both chloride exchange and urea transport was the same. Thus again, urea transport is blocked by compounds that inhibit chloride flux and anion conductance.

Transport of Water and Sorbitol by Oocytes Expressing AE1s. The results reported above indicate that expression of a chloride conductance was accompanied by appearance of an urea transport. Thus, the questions arise whether the conductance pathway is permeable to water and whether other uncharged solutes besides urea are transported similarly.

To test directly whether water can move through the channel, the osmotic water permeability Pf was measured in control water-injected oocytes or in oocytes expressing hUT-B1, tAE1, mAE1, or a frog-specific water channel AQP1 (12). As shown in Fig. 6*A*, water permeability of oocytes injected with tAE1-, mAE1-, and hUT-B1-cRNA did not differ significantly from that of control oocytes, whereas AQP1 increased to 17 times the water membrane permeability. Thus, no measurable water transport was associated with the tAE1-dependent conductance. Moreover, these data confirm that mAE1 (13) and hUT-B1 (11) have no effect on Pf.

We tested then the capacity of anion exchangers to induce the transport of sorbitol. An increase of sorbitol permeability was observed in oocytes expressing tAE1, which was inhibited by niflumic acid, whereas no significant increase occurred in oocytes expressing mAE1 (Fig. 6*B*). Moreover, as for taurine and urea permeabilities, sorbitol permeability was fully abolished by replacement of the tAE1 C terminus by its mouse counterpart (TZM) (data not shown).

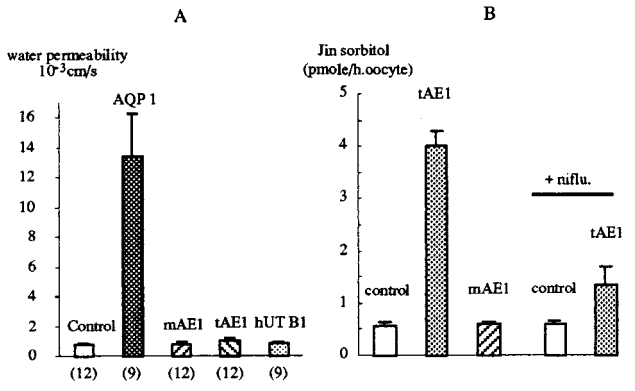

FIG. 6. Permeabilities of tAE1, mAE1, hUT-B1, and AQP1 expressed in oocytes (compare with water-injected control). AQP1 is the water channel expressed in frog urinary bladder (original name FA-CHIP) (12). (*A*) Osmotic water permeability (Pf). Pf is measured by the volumetric method (see *Experimental Procedures*). The number of oocytes measured per group is given in parentheses. (*B*) Sorbitol permeability. Influx was measured in oocytes expressing tAE1 and mAE1, or in water-injected control oocytes, by incubation at 18°C in MBS supplemented with 0.5 mM sorbitol and containing ^{14}C-sorbitol with a constant specific activity of 40.10^3 dpm/nmol sorbitol. The inhibitory effect of niflumic acid ($5{\cdot}10^{-4}$M) was tested on tAE1 and control. (n = 8.)

DISCUSSION

Expression in *Xenopus* oocytes of the trout anion exchanger tAE1 generates a passive taurine transport simultaneously with the expected Cl^- exchange (10). The goal of this study was to determine whether, in the same situation, an increased transport of uncharged organic solutes also could be observed.

First of all, expression of tAE1 induced transport of electroneutral solutes such as sorbitol and urea. In contrast, the mouse anion exchanger mAE1 did not generate such transport, indicating that not all members of the AE1 family behaved similarly. Chimeras constructed from the two homologous proteins tAE1 and mAE1 allowed us to delineate the tAE1 domains involved in the induction of urea transport.

A protein responsible for the facilitated transport of urea in human red blood cells, hUT-B1, recently was cloned (11). A comparison of hUT-B1-mediated and tAE1-induced urea transports reveals several fundamental differences between the two transport mechanisms: (*ii*) tAE1-induced urea permeability was inhibited by anion transport blockers. By contrast, the present results showed that hUT-B1-mediated urea transport remained fully unaffected by these compounds; (*ii*) expression of tAE1 is associated with the induction of an anion conducting pathway (10), and the present data showed a close relationship between tAE1-induced urea permeability and anion conductance: the greater the anion conductance, the greater the urea transport. By contrast, hUT-B1-mediated urea transport is not associated with an increased anion conductance (11); (*iii*) coexpression of hUT-B1 and tAE1 did not lead to functional interaction between them. Taken together, these data indicate that the urea transport generated by expression of tAE1 is related directly with expression of tAE1 and does not occur via stimulation of a putative endogenous urea transporter homologous to UT-B1.

Then the question is to determine whether a relationship exists between this tAE1-dependent urea permeability and the tAE1-dependent taurine transport previously described. (*i*) Both transport systems were induced by expression of tAE1 in oocytes but were not by expression of mAE1; (*ii*) a series of mutants and chimeras constructed from tAE1 and mAE1 indicated that each structural modification introduced in tAE1 had exactly the same consequence for both transport systems: the capacity of tAE1 to generate urea and taurine permeability remained unchanged after deletion of the N-terminal domain or after reduction in the length of the Z-loop; conversely, it was fully abolished by replacement of the tAE1 C-terminal domain by its mouse counterpart; (*iii*) In fact, appearance of both urea and taurine permeabilities clearly were correlated with the expression of an anion conductance. Indeed, tAE1 induces an anion conductance, and the greater the conductance, the greater both urea and taurine permeabilities. By contrast, mAE1 induces neither conductance nor urea and taurine permeabilities. Moreover, any modification in tAE1 structure that does not affect the conductance (deletion of N-terminal domain, reduction of the length of the Z-loop) also did not affect urea and taurine transport. By contrast, structural modifications (swapping of C-terminal domains) abolishing the conductance also abolished both urea and taurine permeability. Finally, it has been observed that conductance, taurine transport, and urea transport were all inhibited by the same anion blockers with the same sensitivity.

These data clearly indicate that expression of tAE1 in oocytes generates an anion conductance and the transport of both taurine and uncharged organic solutes such as urea and sorbitol. Moreover, the present results provided strong evidence that this tAE1-dependent conductance is not through a water channel by which hydrophylic solutes could diffuse because the osmotic water permeability of oocytes was not increased significantly by tAE1 expression.

Because anion exchangers AE1 are considered to mediate electoneutral exchange, the question then arises of the link between tAE1 and the anion conductance induced by expression of tAE1. As previously discussed (14, 15), electrophysiological and pharmacological characteristics of this conductance strongly suggest that it is related directly to the anion exchange protein and would not result from activation of an endogenous channel by the trout band 3 protein. A recent observation, obtained with the patch–clamp technique and performed in trout erythrocytes, supports this view of a direct association between the anion exchanger tAE1 and a channel activity. It has been shown that, in isotonic conditions, trout red blood cells possess a significant DIDS-sensitive Cl^- conductance that is stimulated reversibly by osmotic cell swelling (16). If tAE1 is able to form a conductive pathway mediating the transport of organic solutes, it remains to be seen why the pathway is activated by hypotonic swelling in trout red cells whereas it is constitutively expressed after transfection of tAE1 in the oocytes. The fact that DIDS is unable to block tAE1-mediated chloride exchange in oocytes when it acts as an inhibitor in red cells and that replacement of the Z-loop by its mouse counterpart induces recovery of DIDS sensitivity suggests that tAE1 adopts a different conformation in oocytes and in red cells. In conclusion, it can be suggested reasonably that the trout anion exchanger tAE1 expresses some conductance that is stimulated by red blood cell swelling and is involved in the swelling-activated transport of organic solutes blocked by anion transport inhibitors.

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