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Inhibition of Aquaporin-1 Water Permeability by Tetraethylammonium: Involvement of the Loop E Pore Region

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ABSTRACT

Previously, the only known blockers of water permeability through aquaporin-1 (AQP1) water channels were mercurial reagents such as $HgCl_2$. For AQP1, inhibition by mercury has been attributed to the formation of a mercaptide bond with cysteine residue 189 found in the putative pore-forming region loop E. Here we show that the nonmercurial compound, tetraethylammonium (TEA) chloride, reduces the water permeability of human AQP1 channels expressed in *Xenopus* oocytes. After preincubation of the oocytes for 15 min with 100 μM TEA, AQP1 water permeability was reduced by 20 to 40%, a degree of partial block similar to that obtained with 15 min of incubation in 100 μM $HgCl_2$. The reduction of water permeability was dose-dependent for tested concentrations up to 10 mM TEA. TEA blocks the *Shaker* potassium channel by interacting with a tyrosine residue in the outer pore region. We tested whether an analogous tyrosine residue in loop E of AQP1 could be involved in the binding of TEA. Using polymerase chain reaction, tyrosine 186 in AQP1, selected for its proximity to the mercury-binding site, was mutated to phenylalanine (Y186F), alanine (Y186A), or asparagine (Y186N). Oocyte expression of the mutant AQP1 channels showed that the water permeability of Y186F was equivalent to that of wild-type AQP1; the other mutant channels did not conduct water. However, in contrast to wild-type AQP1, the water permeability of Y186F was not reduced with 100 μM TEA. These results suggest that TEA reduces AQP1 water permeability by interacting with loop E.

Aquaporin-1 (AQP1) is a member of the membrane intrinsic protein (MIP) family of channel-forming proteins. AQP1 and other MIP channels have been shown to function as channels for water (Preston et al., 1992), CO_2 (Nakhoul et al., 1998), ions (Ehring et al., 1990; Weaver et al., 1994; Modesto et al., 1996; Yool et al., 1996; Yasui et al., 1999; Anthony et al., 2000), and other solutes, although the ion channel function of AQP1 remains controversial (Yasui et al., 1999). AQP1 is expressed in kidney, lung, eye, choroid plexus, and red blood cells (Denker et al., 1988; Hasegawa et al., 1993, 1994) where its presence greatly increases membrane permeability to water (Agre et al., 1998). Members of the MIP family are predicted to have six transmembrane domains that are connected by five loops (A–E), with the channel pore thought to be formed by loops B and E (Jung et al., 1994).

AQP1 water channels are inhibited by mercurial compounds such as $HgCl_2$ (Zeidel et al., 1994) via the covalent modification of cysteine 189 in loop E (Preston et al., 1993). Water permeability mediated by AQP1 is reduced by mercury binding, suggesting that cysteine 189 is in the channel pore region. Inhibition by mercury can only be reversed by breaking the covalent bond, for example, with β-mercaptoethanol (Preston et al., 1992).

Our study was initiated to find nonmercurial agents that could be used to block water transport through AQP1. MIP channels and ion channels have six transmembrane domains with putative pore-forming regions found between transmembrane domains 5 and 6 (Jan and Jan, 1992; Jung et al., 1994). Similarities between the general structure of AQP1 and ion channels suggested that ion channel blockers might be evaluated as candidates for novel inhibitors of AQP1 permeability. Different ion channel blockers, including tetraethylammonium (TEA; 0.01 to 10 mM), tetramethylammonium (TMA; 0.5 to 5 mM), tetrapropylammonium (TPA; 0.1 to 5 mM), and clofilium (30 μM) were screened using swelling assays in AQP1-expressing oocytes; only TEA had an appreciable effect on osmotic swelling rate. In these studies we demonstrate that TEA reversibly blocks the water permeability of AQP1 in a dose-dependent manner. Using site-directed mutagenesis we show that the inhibition of water permeability by TEA is influenced by the amino acid sequence of loop E. The fact that TEA sensitivity is removed by

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ABBREVIATIONS: AQP1, aquaporin-1; TEA, tetraethylammonium; TMA, tetramethylammonium; TPA, tetrapropylammonium; MIP, membrane intrinsic protein; RVI, relative volume increase; P_f, osmotic permeability; PCR, polymerase chain reaction.

site-directed mutagenesis of AQP1 confirms that the inhibitory action of TEA is targeted to AQP1 itself and cannot be attributed to nonspecific block of native oocyte channels. This study identifies TEA as a nonmercurial blocker of water transport through AQP1 channels.

Materials and Methods

Preparation of Oocytes and Measurement of Osmotic Permeability (P_f). Oocytes at stages V and VI were harvested from female *Xenopus laevis* and prepared as described previously (Goldin, 1992). Cloned human AQP1 DNA was provided by Dr. P. Agre (Johns Hopkins University, Baltimore, MD). Oocytes were injected the day after isolation with either 50 nl of water or 50 nl of water containing 1 ng of AQP1 RNA. Oocytes were maintained for 2 days at 18°C in ND96P saline (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.6) plus 2.5 mM sodium pyruvate.

Osmotic swelling was performed at 22°C, recorded with a Cohu CCD video camera (Cohu, San Diego, CA), and analyzed by computer using IP Lab Spectrum software (Signal Analytics Corp., Vienna, VA). With images captured every 15 s for 6 min or until the oocytes ruptured. At time 0, oocytes were transferred from 200 mOsM ND96P saline to 100 mOsM ND96P saline, made 50% hypotonic by dilution with an equal volume of distilled water. The area of the oocyte was calculated, converted to estimated volume, and used to calculate the relative volume increase (RVI) and the P_f. P_f of the oocytes was calculated from the time course of osmotic swelling as previously described (Preston et al., 1993). The measured increases in relative volume as a function of time were fit optimally with a second order polynomial, and the initial rates of swelling were calculated between 15 and 75 s, as $d(V/V_o)/dt$, from the linear components of the fits. This measurement was used in the formula $P_f = [V_o \times d(V/V_o)/dt]/[S \times V_w \times (osm_{in} - osm_{out})]$, where initial oocyte volume was $V_o = 9 \times 10^{-4}$, molar ratio of water was $V_w = 18$ cm^3/mol, initial oocyte surface area was $S = 0.045$ cm^2, osmolarity inside the oocyte was estimated as $osm_{in} = 200$ mOsM, and osmolarity outside the oocyte in hypotonic saline was $osm_{out} = 100$ mOsM. The effects of TEA, TMA, TPA, and mercuric chloride on P_f values were investigated by incubating the oocytes in ND96P saline containing the compound for 15 min before each experiment. P_f values are given as mean ± S.E.

Site-Directed Mutagenesis and In Vitro RNA Synthesis. Mutants of AQP1 were generated using an adaptation of the method for rapid site-directed mutagenesis described previously (Landt et al., 1990). Briefly, a fragment between restriction enzyme sites *Eco*RI and *Not*I in the AQP1 expression vector (Preston et al., 1992) was replaced with a cassette containing the point mutation, generated previously by a two-step polymerase chain reaction (PCR) (Horton et al., 1989). Using this method, tyrosine 186 was replaced with either phenylalanine (Y186F), alanine (Y186A), or asparagine (Y186N). The sense primers used in these PCRs were as follows: Y186F primer, 5′-GGCTATTGACTTCACTGGCTGTGGG-3′; Y186A primer, 5′-GGCTATTGACGCCACTGGCTGTGGG-3′; Y186N primer, 5′-GGCTATTGACAACACTGGCT GTGGG-3′. The antisense primer for all reactions was 5′-TACCTAGCATGAACAGATTGGTAATACGACTCACTATA-3′. Mutations were confirmed by DNA sequencing. Capped AQP1 RNA transcripts were synthesized in vitro with T3 RNA polymerase, using *Bam*HI-digested DNA as a template. The RNA was purified by standard phenol-chloroform extraction techniques, and concentration was determined by ultraviolet absorbance and quality checked by agarose gel electrophoresis (Sambrook et al., 1989).

Oocyte Membrane Preparations and Western Blot Analysis. *Xenopus* oocytes expressing wild-type human AQP1 and the Y186F, Y186N, and Y186A mutants were harvested 3 days postinjection, and the membranes from 20 oocytes were isolated as described previously (Geering et al., 1989). Protein concentrations were determined, and 5 μg of each sample were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose. The nitrocellulose blots were probed with anti-AQP1 polyclonal antibodies (Stamer et al., 1995) at a dilution of 1:750 and detected using enhanced chemiluminescence according to manufacturer's instructions (Amersham, Arlington Heights, IL).

Oocyte Immunocytochemistry. Four oocytes expressing AQP1 wild-type and mutant Y186N channels were collected on day 4 of expression and rapidly frozen in Tissue-Tec medium (O.C.T. 4583; Miles, Inc., Tarrytown, NY). Oocytes sections were cut at 8 to10 μm and mounted on glass slides. Sectioned oocytes were postfixed in acetone (100%) for 7 min at 4°C. Preparations were washed in SSC buffer (30 mM sodium chloride, 300 mM sodium citrate) for 10 min at 4°C and then placed in 300 mM glycine for 20 min at 4°C. After a brief rinse in SSC buffer, nonspecific binding was blocked with SSC buffer containing 2% BSA for 1 h at 4°C. The slides were rinsed briefly with SSC buffer and then permeabilized in SSC buffer containing 0.1% Triton X-100 for 1 h at 4°C. Primary anti-AQP1 antibody was generated previously using a carboxy tail fusion protein linked to glutathione *S*-transferase (Stamer et al., 1995). After an overnight incubation with primary antibody (0.5–1.0 mg/ml, 4°C), the slides were washed with SSC buffer and incubated for 1 h at room temperature with secondary antibody (donkey anti-rabbit antibodies tagged with fluorescein isothiocyanate; Sigma, St. Louis, MO) at a dilution of 1:1000. Sections were washed and mounted for viewing on a confocal microscope (TCS-4D; Leica Microsystems Inc., Deerfield, IL).

Results

Oocyte swelling assays were used to identify potential inhibitors of AQP1 water permeability. *Xenopus* oocytes were injected with 1 ng of AQP1 RNA transcripts or were sham-injected with water. Two days later, osmotically induced swelling was measured by transferring oocytes to 50% hypotonic saline (at time 0) and recording the RVI over 225 to 300 s. Figure 1 shows the mean RVIs, averaged for eight oocytes in each treatment group, for AQP1-expressing oocytes (harvested from a single frog) that were preincubated for 15 min in control 100% saline or in 100% saline containing 100 μM TEA or 100 μM $HgCl_2$. Control AQP1-expressing oocytes that were preincubated in saline without TEA showed a rapid osmotically driven increase in relative vol-

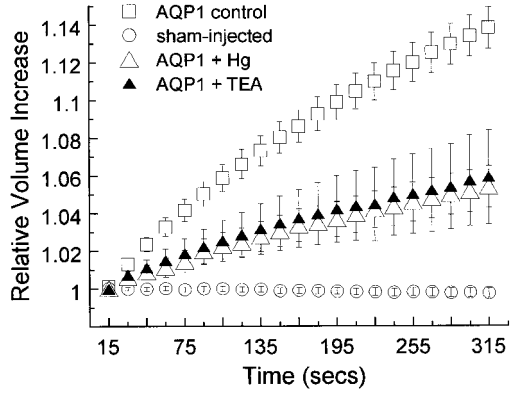

Fig. 1. TEA block of osmotically induced swelling in oocytes expressing human AQP1 channels. RVI was determined using videomicroscopy as described in *Materials and Methods* after the transfer of the oocytes from 100% saline (200 mOsM) into 50% hypotonic saline (100 mOsM) at time 0. Oocytes were preincubated for 15 min either in control saline (□), saline with 100 μM TEA (▲), or 100 μM $HgCl_2$ (△). Sham-injected control oocytes were preincubated in control saline (○). Data were collected, and oocyte areas were calculated using IP Lab Spectrum software. Data show mean ± S.E.; $n = 8$ oocytes for each treatment group.

ume. In contrast, AQP1-expressing oocytes that were preincubated in 100 μM TEA showed a marked reduction in the rate of RVI after their transfer into 50% hypotonic saline, suggesting that TEA inhibited the osmotically induced swelling. The reduction in RVI was similar to the partial block that was produced after 15 min in 100 μM $HgCl_2$. Sham-injected control oocytes did not show any appreciable increases in relative volume with or without TEA treatment.

Increased concentrations of TEA showed a dose-dependent inhibitory effect on the osmotic water permeability of AQP1-expressing oocytes (Fig. 2). Data in Fig. 2 are compiled from a total of 299 oocytes (from 4 to 10 different frogs for each dose); each oocyte was tested with a single dose. At the highest concentration tested, 10 mM TEA reduced water permeability in AQP1-expressing oocytes by an average value of 33% (P_f AQP1 + 10 mM TEA = 63.5×10^{-4} cm/s; cf. P_f AQP1 = 95×10^{-4} cm/s), and in some oocyte preparations, water permeability was reduced by over 60% with 10 mM TEA.

The inhibitory effect of TEA on osmotic water permeability was reversible (Fig. 3). After testing the rate of volume increase after preincubation in 10 mM TEA, the oocyte was rinsed multiple times with control saline and allowed to recover to normal volume over a period of several hours. The same oocyte was then tested again in a swelling assay without TEA. The rapid osmotic swelling response seen in the recovered oocyte demonstrated that the inhibitory effect of TEA was reversible and that the TEA-treated oocyte was expressing a high level of AQP1, equivalent to that of untreated AQP1-expressing oocytes. Reversibility of block was seen in eight of eight oocytes tested with various concentrations of TEA. The estimated affinity of TEA might predict a fast off-rate (much less than several hours); however, it was not possible to evaluate the rate of unblocking of TEA directly. In these experiments, it was essential to allow the oocytes sufficient time to recover to normal volume after the first treatment before testing the swelling response in the absence of TEA.

Site-directed mutagenesis using PCR was performed to investigate whether a tyrosine residue in loop E of AQP1 was involved in mediating the observed inhibitory effect of TEA on water permeability. Tyrosine at position 186, chosen for its proximity to the mercury-sensitive residue (cysteine 189), was mutated to phenylalanine (Y186F), asparagine (Y186N), or alanine (Y186A). RNA synthesized in vitro for each mutant was injected, and the expression of channels in oocyte membranes was verified by Western blot analysis (Fig. 4A). Membrane fractions prepared from wild-type and mutant AQP1-expressing oocytes all contained immunoreactive protein bands, showing that the proteins were expressed in the membrane. Osmotically induced swelling assays demonstrated that Y186F conferred high water permeability comparable to that seen in wild-type AQP1. In contrast, Y186A- and Y186N-expressing oocytes did not show significant increases in relative volume when oocytes were transferred to hypotonic saline. As seen for other nonfunctional AQP1 mutants (Preston et al., 1993), the Y186A and Y186N mutants did not show the higher molecular mass proteins attributed to glycosylated forms of AQP1. Successful expression of mutant Y186N as well as wild-type AQP1 channels in the plasma membrane was confirmed by immunolabeling of cryosectioned oocytes (Fig. 4B).

The effect of TEA on the water permeability of Y186F-expressing oocytes was evaluated by osmotic swelling assays in 50% hypotonic saline. Figure 5 shows the RVI for Y186F-expressing oocytes (harvested from a single frog) that were preincubated in either control saline or in saline containing 100 μM TEA or 100 μM $HgCl_2$. Y186F-expressing oocytes preincubated in control saline showed a rapid increase in relative volume. The magnitude of this increase in relative volume was similar to that observed for wild-type AQP1-expressing oocytes (shown in Fig. 1). When preincubated in 100 μM TEA for 15 min, Y186F-expressing oocytes showed a comparably rapid increase in relative volume with no evidence of TEA inhibition. The rate and magnitude of the RVIs in Y186F-expressing oocytes were unaffected by TEA pretreatment at 100 μM (Fig. 5); a slight reduction in Y186F swelling rate at 10 mM TEA was not significant (data not shown). However, as expected, the inhibitory effect of $HgCl_2$ was not impaired. Y186F-expressing oocytes showed a reduction in RVI with $HgCl_2$ that was similar in magnitude to that observed with wild-type AQP1-expressing oocytes. Sham-injected control oocytes were resistant to osmotically induced swelling and were unaffected by the presence of TEA.

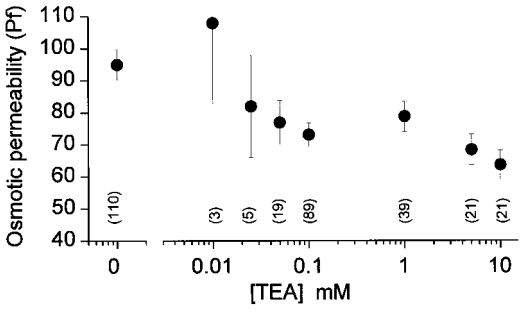

Fig. 2. Dose-response relationship for the effect of TEA on P_f of oocytes expressing wild-type AQP1. Oocytes expressing wild-type AQP1 channels were preincubated for 15 min in saline containing TEA at the indicated concentrations. RVI was monitored every 15 s after the transfer of each oocyte into 50% hypotonic saline (100 mOsM) containing the same respective concentration of TEA. Data were collected, and oocyte areas were calculated using the IP Spectrum software. Data are given as mean ± S.E. Final osmolarity was controlled in the TEA solutions by substituting NaCl with equimolar TEA chloride.

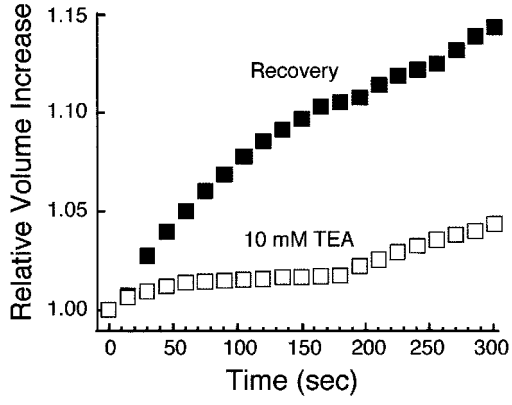

Fig. 3. Reversible block by 10 mM TEA of osmotically driven swelling in an AQP1-expressing oocyte. An oocyte expressing wild-type AQP1 channels was preincubated for 15 min in saline with 10 mM TEA. The RVI was assessed after the transfer of the oocyte into 50% hypotonic saline (100 mOsM containing 10 mM TEA) at time 0 (□). The oocyte was removed from the hypotonic saline, allowed to recover during several rinses in control saline for 4 h, and then the swelling response was repeated without TEA in 50% hypotonic saline (■).

Osmotic water permeability (P_f) values were calculated for oocytes expressing wild-type or mutant AQP1 channels and for sham-injected oocytes. The results shown in Fig. 6 are compiled from oocytes taken from 4 to 10 different frogs. Y186F-expressing oocytes showed an osmotic water permeability ($P_f = 97 \pm 5 \times 10^{-4}$ cm/s) similar to that of wild-type AQP1 ($P_f = 95 \pm 5 \times 10^{-4}$ cm/s). Expression of Y186A and Y186N mutants yielded values for osmotic water permeabilities ($P_f = 1.4 \pm 0.8 \times 10^{-4}$ cm/s and $P_f = 5.5 \pm 3 \times 10^{-4}$ cm/s, respectively) similar to that of sham-injected oocytes ($P_f = 3.8 \pm 0.2 \times 10^{-4}$ cm/s). Preincubation in 100 μM TEA significantly reduced the osmotic water permeability of wild-type AQP1 channels (P_f with 100 μM TEA $= 73 \pm 4 \times 10^{-4}$ cm/s; $P < .001$) but had no significant effect on the osmotic water permeability of Y186F mutant channels (P_f with 100 μM TEA $= 100 \pm 8 \times 10^{-4}$ cm/s; $P > .05$). Water permeability values of both wild-type and Y186F AQP1 channels were reduced significantly by 100 μM mercury ($P_f = 63 \pm 6 \times 10^{-4}$ cm/s and $P_f = 60 \pm 5 \times 10^{-4}$ cm/s, respectively; $P < .001$). After 15 min in 100 μM $HgCl_2$, the block of AQP1 water permeability is only partial; a higher concentration produces a greater degree of block. At 300 μM $HgCl_2$, the P_f value was $11 \pm 4 \times 10^{-4}$ cm/s ($n = 12$), showing 83% block of wild-type AQP1 water permeability (P_f without mercury $= 66 \pm 8 \times 10^{-4}$ cm/s; $n = 20$). Significance levels were determined by two-tailed Student's t test. In contrast to TEA, two other quaternary ammonium derivatives, TMA and TPA, were not effective in inhibiting wild-type AQP1 water permeability (Table 1).

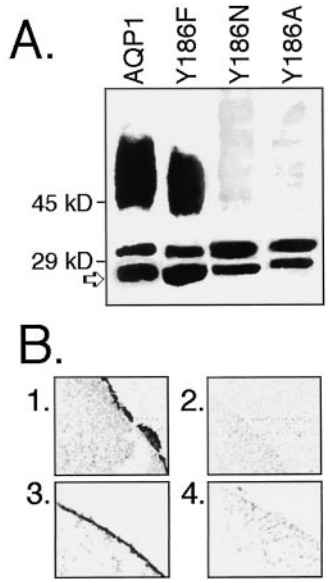

Fig. 4. Western blot and immunocytochemical analyses of channel expression in plasma membranes of oocytes injected with RNA encoding wild-type or mutant (Y186F, Y186A, Y186N) AQP1 channels. A, Western blot analysis. Oocytes were injected with 1 ng of RNA and each lane was loaded with 5 μg of total membrane protein prepared from 20 oocytes. The positions of the 45- and 29-kDa molecular mass markers are indicated on the left. The arrow shows the position of the native 28-kDa protein, whereas the higher molecular mass species represents different glycosylated species of AQP1 as described previously (Preston et al., 1993). B, confocal immunofluorescence microscopy was used to visualize the expression of AQP1 wild-type and Y186N proteins in the plasma membrane of *Xenopus* oocytes labeled with antibodies generated against a fusion peptide from the carboxy tail sequence of AQP1 (Stamer et al., 1995) and tagged with fluorescent secondary antibodies. Fixation and immunolabeling procedures are described in *Materials and Methods*. The panels show pairs of serial sections (10 μm) from oocytes expressing AQP1 wild-type (1, 2) or Y186N (3, 4) proteins. Staining is evident in sections labeled with primary and secondary antibodies (1, 3) and reduced by prior incubation of the primary antibody with AQP1 fusion protein (2, 4), indicating that the observed binding is specific for the AQP1 epitope.

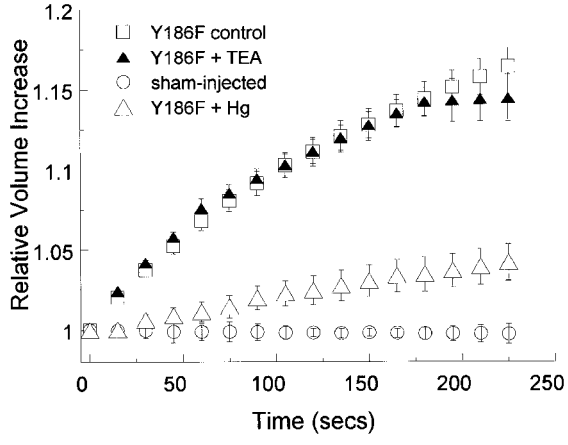

Fig. 5. Lack of effect of TEA on the osmotically induced swelling of oocytes expressing Y186F mutant AQP1 channels. Oocytes were preincubated for 15 min in control saline (□), saline with 100 μM TEA (▲), or 100 μM $HgCl_2$ (△). Sham-injected control oocytes were preincubated in control saline (○). Osmotic swelling was monitored every 15 s after the transfer of the oocytes into 50% hypotonic saline (100 mOsM) at time 0. Data were collected, and oocyte areas were calculated using the IP Spectrum software. Data show the mean ± S.E.; $n = 8$ for each treatment group.

Discussion

Using a standard oocyte swelling assay, we found that TEA, at a relatively low concentration (100 μM), significantly inhibited the water permeability of AQP1-expressing oocytes. The inhibitory effect of TEA on AQP1 water permeability was dose-dependent and reversible. A tyrosine residue at position 186 is located by transmembrane topology models as being

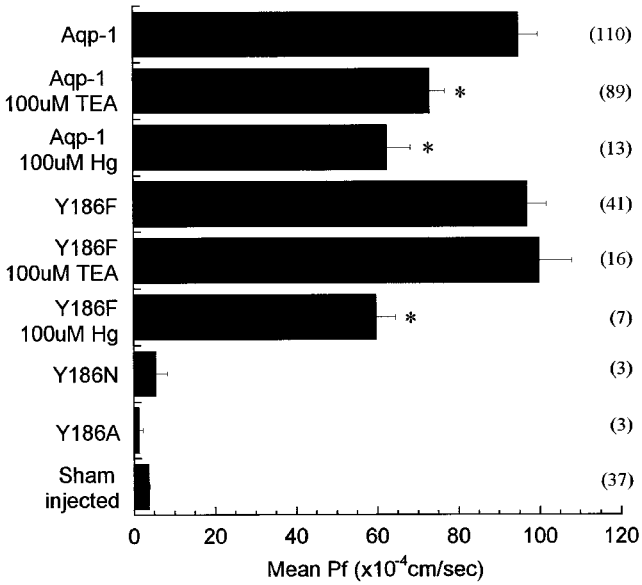

Fig. 6. Summary of the P_f values for AQP1 wild-type and mutant channels and the effects of TEA and $HgCl_2$. Oocytes were injected with 1 ng of RNA, and osmotic water permeabilities were measured at 2 days postinjection by transfer of the oocytes into 50% hypotonic medium. Data represent the mean ± S.E.; n values are shown in parentheses. Student's t tests were used to analyze the results. An asterisk (*) indicates values that are significantly different ($P < .001$) from wild-type AQP1.

near the external side of the pore-forming region of the channel. Mutagenesis of this residue reduced the sensitivity of AQP1 channels to block caused by external TEA, demonstrating that the inhibitory effect of TEA is mediated directly by AQP1, not by native oocyte channels, and that the loop E region is involved directly or indirectly in forming the TEA binding site.

TEA is known as an open channel blocker of K^+ channels (Armstrong, 1990). The TEA sensitivity of *ShakerB* K^+ channels correlates with the nature of the amino acid at position 449 (MacKinnon and Yellen, 1990; Hopkins et al., 1994). Substitution of threonine 449 by tyrosine increases the sensitivity of the K^+ channel to TEA by 40-fold, from an IC_{50} value of ~25 to ~0.6 mM (MacKinnon and Yellen, 1990). Threonine 449 is located in the K^+ channel S5-S6 loop (connecting the fifth and sixth transmembrane domains), which contains the sequence GYG that contributes to the ion selectivity filter (Doyle et al., 1998). The analogous region in loop E of AQP1 is proposed to form part of the water pore (Preston et al., 1993) and the solute selectivity filter (Lagree et al., 1999) and coincidentally contains the cysteine binding site for mercury within the sequence GCG. Mutations in AQP1 loop E that changed cysteine 189 to methionine, tryptophan, or tyrosine reduced water permeability by as much as 95%, whereas substitution of cysteine 189 with alanine or serine did not affect water permeability but removed inhibition by $HgCl_2$ (Preston et al., 1993).

The tyrosine residue at position 186 in AQP1 loop E was chosen for mutagenesis because of its proximity to the AQP1 mercury binding site (cysteine 189). Results of our analyses of water permeability suggested that tyrosine 186 is analogous but not identical with the aromatic binding site for TEA that has been described for K^+ channels. The binding sites in the two classes of channels showed similarities in the relative potencies of the tetraalkylammonium agents with TEA being more effective than TPA or TMA in the *Shaker* wild-type K^+ channel (Heginbotham and MacKinnon, 1992), more effective than TPA on native neuroblastoma-delayed rectifier K^+ currents (Quandt and Im, 1992), and more effective than TPA or TMA in AQP1-expressing oocytes (Table 1). In contrast, the TEA binding sites in the K^+ and AQP1 channels differed in sensitivity to the nature of the substituted residue. In K^+ channels, substitution of tyrosine with phenylalanine did not impair TEA binding; the aromatic rings have been proposed to form the TEA binding site (Heginbotham and MacKinnon, 1992). In contrast, we show in AQP1 that the substitution of tyrosine 186 with phenylalanine reduced the sensitivity of AQP1 water permeability to block by TEA. These data suggest that the hydroxyl group on tyrosine may contribute to the putative TEA binding site in AQP1 channels.

Mutations of AQP1 tyrosine 186 to alanine or asparagine created channels that were nonfunctional with respect to water permeability. The AQP1 Y186N mutant also was nonfunctional with respect to regulated ionic permeability (Anthony et al., 2000), although the protein was translated and transported to the oocyte plasma membrane (Fig. 4). Although impermeable to water and ions, Western blot and immunocytochemical analyses indicated that Y186N mutant channels were expressed in the oocyte plasma membrane. Therefore, the absence of water permeability is not explained by a gross failure of expression and targeting of the channels to the oocyte membrane. More subtle effects of the mutations on disrupting protein folding and structure cannot be ruled out. Although the glycosylation patterns seen by Western blot were different for the nonfunctional mutants, improper glycosylation may not explain the loss of function. AQP1 with valine substituted for cysteine at position 189 was shown not to be glycosylated by Western blot analysis, but it was permeable to water when expressed in oocytes (Preston et al., 1993). The cause of the loss of function in AQP1 Y186N and Y186A remains to be determined but indicates that AQP1 channel structure is sensitive to alterations in this position.

The results of this study demonstrate that AQP1 function can be modified by pharmacological agents other than mercurial compounds. TEA, unlike mercury, is a reversible blocker that does not require treatment with reducing agents. This observation is an exciting one because it may identify a potential lead compound for development of drugs for therapeutic intervention in clinical disorders that could be associated with disturbances in aquaporin function, such as glaucoma, pulmonary edema, and autosomal dominant polycystic kidney disease (King and Agre 1996). AQP1 water channels are abundantly expressed in renal tubules (Knepper et al., 1996) and mediate the characteristically high water permeability of the tubules required for efficient near-isosmolar fluid absorption (Schnermann et al., 1998). Further studies on the effects of TEA on transmembrane water transport in renal systems will be of interest, particularly because TEA is used commonly as a classical substrate for studies of organic cation transport in renal tubules. The K_m for organic cation transporters in renal tubules is 0.1 mM TEA (Ullrich et al., 1991), a concentration that we show to be effective in inhibiting up to 30% of AQP1 water permeability. Our data present a novel characterization of a nonmercurial blocker of water transport through AQP1 channels. The block of water permeability by TEA is dose-dependent, reversible, and influenced directly by a tyrosine residue in the amino acid sequence of loop E.

TABLE 1

Water permeability values (P_f) for wild-type AQP1-expressing oocytes tested with different quaternary ammonium compounds and paired untreated AQP1-expressing oocytes from the same batches of oocytes

Compound	Conc.	P_f[a]	Conc.	P_f[a]
	mM		*mM*	
TEA	0	95 ± 5 (110)	0.1	73 ± 4 (89)
TPA	0	60 ± 10 (14)	0.1	64 ± 16 (10)
	0	47 ± 8 (6)	1	48 ± 5 (4)
	0	70 ± 16 (8)	5	78 ± 38 (4)
TMA	0		0.5	92 ± 24 (4)
	0	98 ± 10 (4)	1	111 ± 20 (4)
	0		5	97 ± 41 (4)

Conc., concentration.
[a] P_f values (units × 10^{-4} cm/s); mean ± S.E. (*n*).

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