

Expression of Multiple Water Channel Activities in *Xenopus* Oocytes Injected with mRNA from Rat Kidney

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ABSTRACT To test the hypothesis that renal tissue contains multiple distinct water channels, mRNA prepared from either cortex, medulla, or papilla of rat kidney was injected into *Xenopus* oocytes. The osmotic water permeability (P_f) of oocytes injected with either 50 nl of water or 50 nl of renal mRNA (1 $\mu\text{g}/\mu\text{l}$) was measured 4 d after the injection. P_f was calculated from the rate of volume increase on exposure to hyposmotic medium. Injection of each renal mRNA preparation increased the oocyte P_f . This expressed water permeability was inhibited by *p*-chloromercuriphenylsulfonate and had a low energy of activation, consistent with the expression of water channels. The coinjection of an antisense oligonucleotide for CHIP28 protein, at an assumed >100-fold molar excess, with either cortex, medulla, or papilla mRNA reduced the expression of the water permeability by ~70, 100, and 30%, respectively. Exposure of the oocyte to cAMP for 1 h resulted in a further increase in P_f only in oocytes injected with medulla mRNA. This cAMP activation was not altered by the CHIP28 antisense oligonucleotide. These results suggest that multiple distinct water channels were expressed in oocytes injected with mRNA obtained from sections of rat kidney: (a) CHIP28 water channels in cortex and medulla, (b) cAMP-activated water channels in medulla, and (c) cAMP-insensitive water channels in papilla.

INTRODUCTION

It has been postulated for many years that water channels exist in renal epithelia, but until recently this view rested mainly upon indirect evidence such as the vasopressin-dependent appearance of intramembranous aggregophores (for review see Harris,

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Strange, and Zeidel, 1991) or the biophysical characterization of transepithelial water movement. The latter studies were carried out on isolated perfused tubules (Berry, 1983; Whitttembury, Carpi-Medina, Gonzalez, and Linares, 1984), isolated intact epithelial cells (Carpi-Medina, Leon, Espidel, and Whitttembury, 1988; Echevarria, Gutierrez, Gonzalez, and Whitttembury, 1991), or membrane vesicles (Van Heeswijk and Van Os, 1986; Verkman, Lencer, Brown, and Ausiello, 1988), and were based upon measurements of osmotic (P_f) and diffusive (P_d) water permeabilities, the effect of mercurial compounds on P_f and P_d , and measurements of the energy of activation (E_a) of water movement. A physiological characterization of water transfer across different segments of the nephron has also emerged. Besides well-described quantitative differences in segmental P_f values, it is now known that water movement across the proximal tubule wall is not hormonally controlled (Verkman, 1989). In contrast, the water permeability of the apical membrane of collecting duct is regulated by vasopressin (Harris et al., 1991), and in the papillary collecting duct, also by a vasopressin-independent process elicited *in vivo* by water deprivation (Lankford, Chou, Terada, Wall, Wade, and Knepper, 1991).

Attempts to characterize renal epithelial water channels at the molecular level have generally followed two different lines of experimental approach. First, proteins (ranging between 17 and 55 kD) that appear within the apical membrane of epithelial cells from the amphibian urinary bladder during antidiuretic hormone (ADH) stimulation have been isolated (Harris et al., 1991; Valenti, Calamita, and Svelto, 1991), and antibodies against these proteins have been shown to inhibit ADH-induced water permeability (Valenti et al., 1991). Second, water channels have been expressed in *Xenopus* oocytes after the injection of kidney mRNA (Milovanovic, Chao, Frindt, and Windhager, 1989; Zhang, Logee, and Verkman, 1990). In addition, CHIP28 protein has been isolated from red cells and kidney (Denker, Smith, Kuhajda, and Agre, 1988) and characterized (Smith and Agre, 1991). The cDNA for human erythrocyte CHIP28 protein, isolated by Preston and Agre (1991), confers water channel activity when expressed in *Xenopus* oocytes (Preston, Carrol, Guggino, and Agre, 1992) or when pure CHIP28 protein is reconstituted in proteoliposomes (Zeidel, Ambudkar, Smith, and Agre, 1992). CHIP28 immunoreactivity was demonstrated in apical and basolateral membranes of proximal tubules and descending limbs of Henle's loop but not in distal tubules or in collecting ducts (Nielsen, Smith, Christensen, Knepper, and Agre, 1993).

To test the hypothesis that the nephron contains multiple water channels which are distinct from each other and vary in abundance in different nephron segments, we set out to express water channel activity in *Xenopus* oocytes injected with mRNA prepared from three areas of the rat kidney: cortex, whole medulla, and papilla. To characterize the expressed putative water channels, we have (a) measured the effect of temperature (E_a) and of a mercurial compound on the P_f of oocytes, (b) coinjected renal mRNA with an antisense oligonucleotide for CHIP28, and (c) examined the effect of cAMP on the water permeability of the injected oocytes.

MATERIALS AND METHODS

Total RNA was isolated separately from superficial cortex, whole medulla, and papilla of Sprague-Dawley rat kidneys using the RNazol method (Cinna/Biotecx Laboratories, Friends-

wood, TX) (Chomczynski and Sacchi, 1987). Papilla included only the first 4 mm of tissue from the papillary tip. Poly(A⁺) RNA was separated with an oligo (dT)-cellulose column (Badley, Bishop, St. John, and Frelinger, 1988). The human cRNA for CHIP28, prepared as described by Preston et al. (1992), was used in some series of experiments. Two mouse CHIP28 oligonucleotide primers were used: one antisense for +16 to +40, and the other sense for +14 to +38. The sequence for the antisense oligonucleotide is 5'-CAGCCCTCCAGAAGAGCTTCTTCTT-3', and the sequence for the sense oligonucleotide is 5'-TCAAGAAGAAGCTCTTCTGGAGGGC-3'.¹ These primers were synthesized on a model 380B DNA synthesizer (Applied Biosystems, Inc., Foster City, CA) and purified by reverse-phase HPLC on a model 152 separation system (Applied Biosystems, Inc.).

Preparation and Injection of Oocytes

Oocytes (stages V and VI) were prepared as described previously (Milovanovic, Frindt, Tate, and Windhager, 1991). Briefly, portions of the ovaries of mature *Xenopus laevis* were removed under anesthesia (immersion in 0.2% ethyl ester 3-aminobenzoic acid) and small clumps of oocytes were separated out with fine forceps in Ca-free Barth's solution. Oocytes were then defolliculated by gentle agitation for 60–90 min in Ca-free Barth's solution containing 2 mg/ml each of collagenase (type IA; Sigma Chemical Co., St. Louis, MO) and hyaluronidase (type III; Sigma Chemical Co.). Subsequently, the oocytes were extensively washed and allowed to recover overnight in modified Leibovitz's L-15 medium (GIBCO BRL, Bethesda, MD). We modified this medium by diluting it with 1 vol of distilled water, adding 15 mM HEPES, 5 mM L-glutamine, and 100,000 U/liter of penicillin-streptomycin, and adjusting the pH to 7.4 with NaOH.

Each oocyte was injected with either 50 nl of water or 50 nl of mRNA solution. The mRNA injected was either renal poly(A⁺) RNA (50 ng) or CHIP28 cRNA (0.2 ng). In some experiments the renal mRNA preparations (40 ng) or CHIP28 cRNA (0.2 ng) were coinjected with either sense (2.27 ng) or antisense (2.27 ng) oligonucleotide primers. The sense or antisense oligonucleotides were mixed with either mRNA or cRNA and the mixture was warmed to 65°C for 2 min and then cooled down to room temperature just before injection.

Oocytes were injected by means of a pressure system (Picospritzer II; General Valve Corp., Fairfield, NJ) using micropipettes with beveled tips of 18–20 μm external diameter. The injected oocytes were kept in modified Leibovitz's medium at 20°C in an incubator for 4 d before measuring their P_f .

Measurement of Oocyte P_f

The method used was a modification of that described by Fischbarg, Kuang, Vera, Arant, Silverstein, Loike, and Rosen (1990). P_f was calculated from the time course of the change in oocyte volume upon exposure to a hyposmotic bathing solution. For this purpose, one oocyte was superfused in a 0.3 ml lucite chamber at 3–4 ml/min. The oocyte was loosely attached to the glass bottom of the chamber with Cell-Tak (Collaborative Research Inc., Bedford, MA) and viewed with an inverted microscope equipped with a video camera. The objective lens of the microscope had a magnification of 4× and an n.a. of 0.13. The total magnification on the monitor screen was 92×. An image frame of the oocyte in sharp focus was recorded every 20 s by a frame grabber and auxiliary processing board in a computer with math coprocessor. The image was processed by a transfer function so that pixels within the oocyte appeared black and

¹ During the process of editorial revision the nucleotide sequence for rat parotid CHIP28 cDNA became available (Dai, Y., J. Li, and B. J. Baum, 1992 in Genbank accession LO7268). The antisense and sense nucleotides we used showed 100% homology to the rat cDNA.

those on the outside appeared white. A rapid pixel summation was performed to determine oocyte area in the focal plane. Simultaneously, the computer calculated the oocyte volume, assuming that the oocyte is a sphere without microvilli. P_f was calculated according to:

$$P_f = dV/dt[1/(A \cdot \Delta\pi)]$$

where A is the area, V is the volume, and $\Delta\pi$ is the osmolality gradient at zero time. dV/dt is the slope of the linear fit in the plot of oocyte volume vs. time in hyposmotic solution. $\Delta\pi$ was expressed as water concentration and was calculated from $[(55.6 - 0.014) - (55.6 - 0.178)]/55.6$, where 55.6 is the molar concentration of water, 0.178 is the molar salt concentration assumed to be isosmotic to intracellular fluid of the oocyte, and 0.014 is the molar salt concentration of extracellular fluid. Fig. 1 shows two typical determinations of P_f . The squares correspond to volume measurements in one oocyte injected with water, and the circles correspond to measurements in an oocyte injected with mRNA. During the initial 80 s we

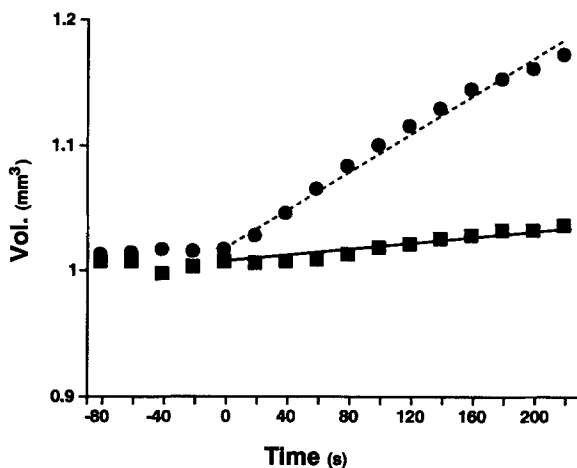


FIGURE 1. Calculation of P_f in two oocytes. Oocyte volume (plotted on the ordinate) was measured at room temperature, while the oocyte was superfused with Barth solution. After the initial 80 s, corresponding to time zero on the abscissa, the superfusate was changed from isosmotic to hyposmotic Barth solution. The oocyte volume was constant in isosmotic solution but increased in a linear fashion after the change to the hyposmotic solution. The measurements shown correspond to a water-injected (squares) and to a papilla mRNA-injected oocyte (circles); the P_f values were 11.9 and 50.9 $\mu\text{m/s}$, respectively.

superfused the oocyte with isosmotic Barth solution (178 mosmol/kg) and then changed to a hyposmotic solution (14 mosmol/kg) for the subsequent 220 s. The hyposmotic solution was identical to the isosmotic solution except for the removal of 88 mM NaCl. The oocyte volume was constant during the perfusion with isosmotic solution and increased in quasi-linear fashion while exposed to the hyposmotic solution. In the example of Fig. 1, the P_f values calculated were 11.9 $\mu\text{m/s}$ for the water-injected oocyte and 50.9 $\mu\text{m/s}$ for the oocyte injected with mRNA.

In some groups of oocytes P_f was measured after treatment with either 8-(4-chlorophenylthio)-adenosine 3':5'-cyclic monophosphate (Cl-PheS-cAMP; Sigma Chemical Co.), or sodium *p*-chloromercuriphenylsulfonate (pCMBS).

To calculate the percentage change in expressed P_f of groups of oocytes resulting from the treatment with drugs or coinjection with antisense oligonucleotide, we subtracted the P_f value of water-injected oocytes from the P_f value of treated and nontreated mRNA-injected oocytes.

Measurement of Oocyte Conductance

Oocytes were superfused with isotonic Barth solution and impaled with two glass microelectrodes filled with 2 M KCl. The electrode resistance was $\sim 5 \text{ M}\Omega$. Once the membrane potential was recorded, the membrane voltage was clamped at -50 mV using a TEV-200 voltage clamp amplifier (Dagan Corp., Minneapolis, MN). The currents were recorded with a strip chart recorder. Oocyte membrane conductance was calculated from the change in current caused by a 10-mV pulse. The membrane conductance was measured both with isosmotic superfusate and 3 min after changing the superfusate to a hyposmotic solution. The composition of these two solutions was identical to those used for P_f measurements.

Statistical Analysis

Where three or more groups of oocytes were compared, analysis of variance was used to obtain the degree of significance. Otherwise, the Student's *t* test was used.

RESULTS

Effect of Injection of mRNA from Cortex, Medulla, and Papilla

We injected oocytes with mRNA from either cortex, medulla, or papilla. P_f of six to eight oocytes was measured in each condition in each experiment. The three mRNA preparations increased P_f compared with the low value measured in water-injected oocytes (or noninjected oocytes). The average P_f values (in micrometers per second) were 14.5 ± 0.7 in 66 water-injected oocytes, 31.6 ± 1.9 in 47 cortex mRNA-injected oocytes, 40.9 ± 1.7 in 84 medulla mRNA-injected oocytes, and 69.3 ± 4.5 in 16 papilla mRNA-injected oocytes. Medulla and papilla mRNA produced a larger increase in P_f than cortex mRNA. These results suggest that the three different areas of the kidney contain a message (or messages) for water channels that increases the oocyte water permeability.

Effect of pCMBS

Next we examined the effect of pCMBS on the expressed P_f . This compound reacts with sulfhydryl groups and is known to inhibit the water permeability of cells containing water channels (Macey and Farmer, 1970; Whittembury et al., 1984). Oocytes injected with water or renal mRNA were incubated in medium containing 1 mM pCMBS 30–40 min before and during the measurements of P_f . pCMBS had no effect on the P_f of the water-injected oocytes, but inhibited 50–83% of the water permeability expressed after the injection of kidney-derived mRNA (Fig. 2A), consistent with the presence of water channels. Similar results have been reported by others (Hoch, Gorfien, Linzer, Fusco, and Levine, 1989; Zhang et al., 1990). These results indicate that the increased P_f of the oocytes injected with renal mRNA is accounted for by the expression of membrane proteins containing sulfhydryl groups.

We measured the oocyte membrane conductance to examine whether the reduced rate of swelling of pCMBS-treated oocytes could be accounted for by a loss of intracellular solutes leading to a reduction in the osmotic gradient across the cell membrane. The effect of pCMBS was evaluated under conditions identical to those used to measure the effect of this compound on P_f . In water-injected oocytes pCMBS did not significantly alter the membrane conductance in isosmotic or hyposmotic

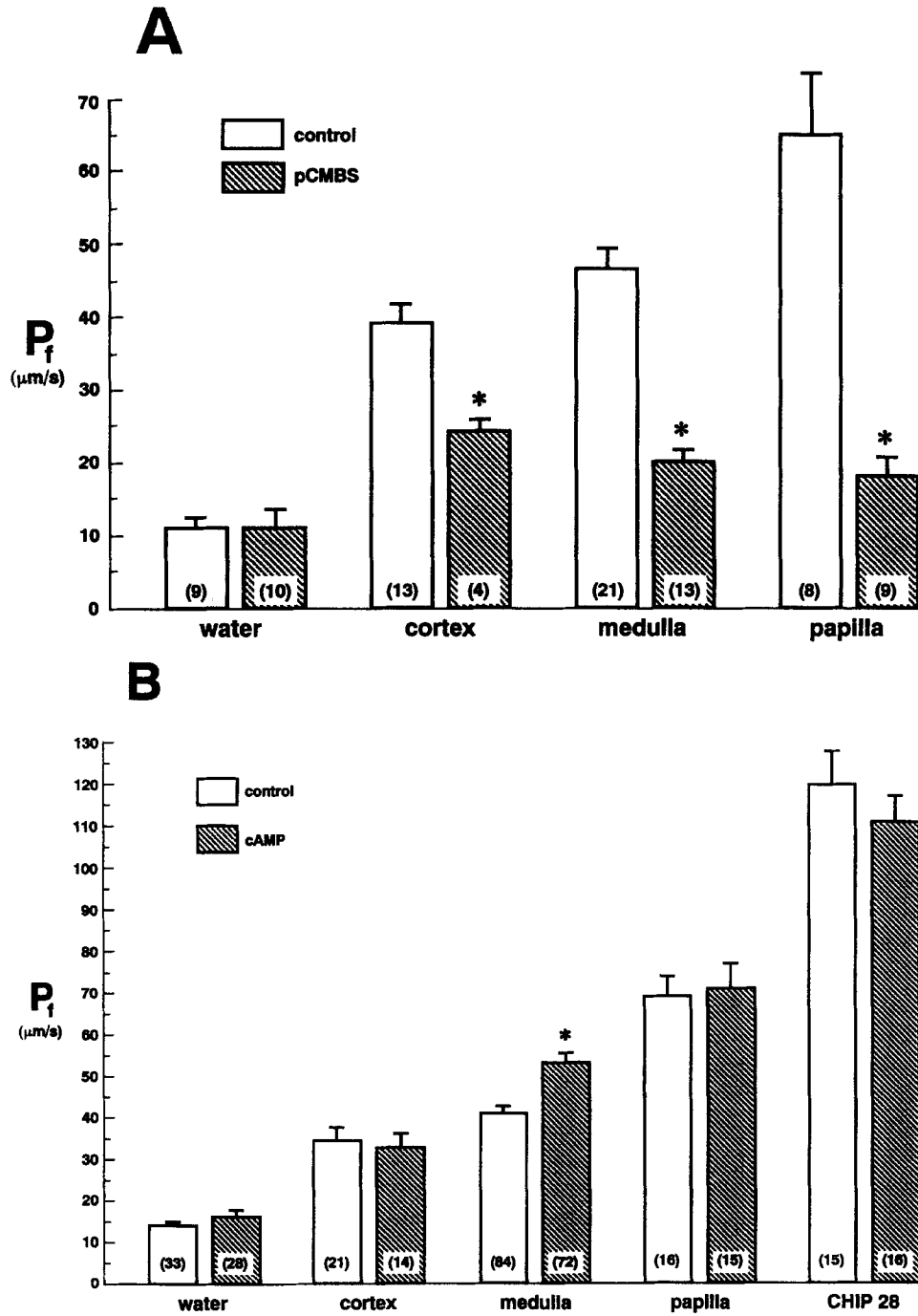


FIGURE 2.

solutions. In medulla mRNA-injected oocytes, pCMBS caused a significant increase in membrane conductance in isosmotic (1.0 ± 0.04 to $2.4 \pm 0.2 \mu\text{S}$) and hyposmotic (0.8 ± 0.1 to $1.8 \pm 0.2 \mu\text{S}$) solutions. These results indicate that pCMBS only caused a small increase in membrane leakiness in mRNA-injected oocytes. Assuming an intracellular K^+ concentration of 100 mM and using the measured values of membrane voltage (mean value = -57 ± 7 mV, $n = 7$ oocytes) and conductance in the presence of pCMBS, one can estimate that the K^+ loss attributable to pCMBS during 3 min of exposure to the hyposmotic solution would be $\sim 10^{-10}$ mol. Since this is $< 1\%$ of the oocyte K^+ content, it is very unlikely that the osmotic gradient was altered significantly by pCMBS. Therefore, the reduction of the rate of swelling in

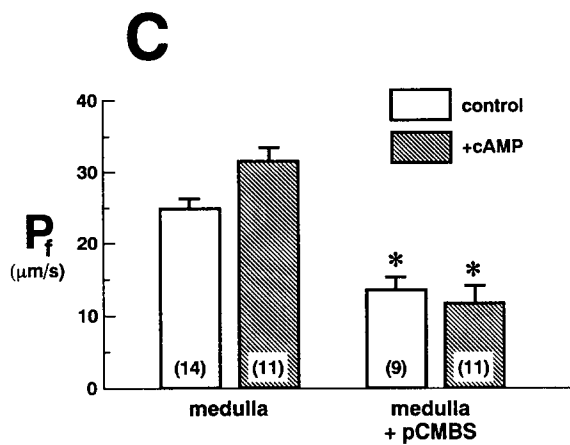


FIGURE 2. Effect of pCMBS or Cl-PheS-cAMP on the expressed water permeability. Bars represent the mean \pm SE of P_f in groups of oocytes injected with water or renal mRNA. The number of oocytes in each group is given in parenthesis. (A) pCMBS inhibited the water permeability expressed after the injection of renal mRNA. Where indicated, the oocytes were incubated in the presence of 1 mM pCMBS before (30 min) and during the measurements of P_f . Asterisks

indicate a value significantly different from the untreated control ($P < 0.001$). (B) Effect of Cl-PheS-cAMP. Where indicated, the oocytes were exposed to 10^{-4} M Cl-PheS-cAMP for 1 h before and during the P_f measurements. In oocytes injected with medulla mRNA P_f increased with Cl-PheS-cAMP ($*P < 0.001$), but no effect was observed in oocytes injected with either water, cortex mRNA, papilla mRNA, or CHIP28 cRNA. (C) pCMBS inhibition of the stimulatory effect of cAMP in medulla mRNA-injected oocytes. Oocytes treated and not treated with Cl-PheS-cAMP were preincubated with 1 mM pCMBS before (30 min) and during the P_f measurements. See text for details. Asterisks indicate values significantly different from the corresponding group not treated with pCMBS ($P < 0.005$).

pCMBS-treated oocytes is due to a decrease in the water permeability of expressed water channels.

Effect of Cl-PheS-cAMP

Fig. 2 B shows the effect of cAMP on water permeability expressed in oocytes. In these experiments oocytes were exposed for 1 h to isosmotic bathing solution containing 10^{-4} M Cl-PheS-cAMP before measuring P_f . This cAMP analogue was also present in the solutions during the P_f measurements. There was no detectable effect of cAMP on the P_f of oocytes injected with either water or mRNA prepared from cortex or papilla. In contrast, exposure to cAMP significantly ($P < 0.001$) increased

by 30% the water permeability of the oocytes injected with medulla mRNA. In addition, we examined the effect of pCMBS on the Cl-PheS-cAMP-stimulated water permeability of oocytes injected with medulla mRNA. The results are summarized in Fig. 2 C. The water permeability of the oocytes, either treated with Cl-PheS-cAMP or untreated, was reduced to essentially the same P_f value by pCMBS. Therefore, pCMBS abolished the cAMP effect, suggesting that the Cl-PheS-cAMP-dependent water permeability also resides in a membrane protein containing a critical sulfhydryl group.

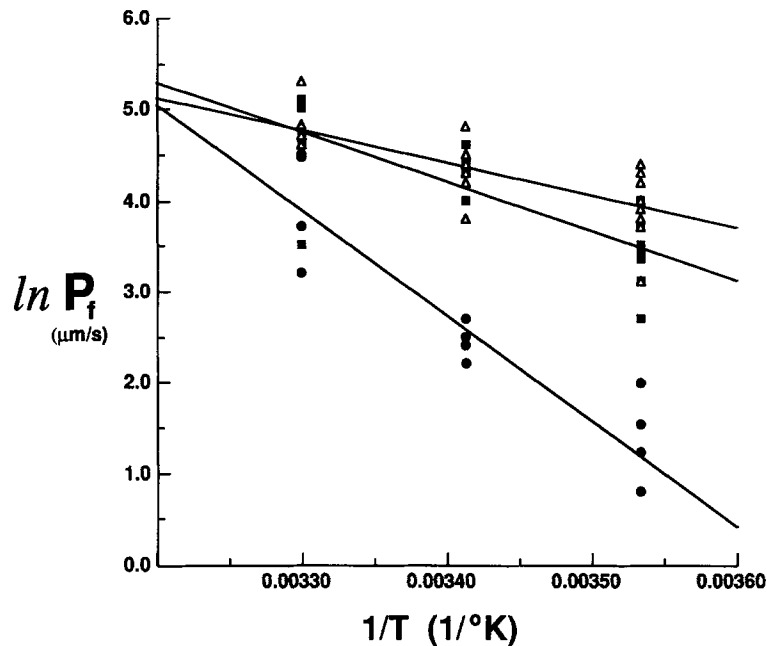


FIGURE 3. Arrhenius plots of the natural logarithm of P_f values vs. the reciprocal of the temperature in Kelvin degrees. P_f was measured in separate groups of oocytes at 10, 20, and 30°C. Each symbol represents a single oocyte. The activation energy for the water-injected oocytes (filled circles) was 22 kcal/mol. In medulla mRNA-injected oocytes E_a was 10 kcal/mol in the absence of Cl-PheS-cAMP (squares) and 6.8 kcal/mol with CL-PheS-cAMP (triangles). Points in each group were fitted to a linear regression function.

Activation Energy of P_f

A criterion commonly used to differentiate whether water moves by diffusion through the lipid bilayer or via water channels is the measurement of the activation energy (E_a) of P_f (Finkelstein, 1987). To estimate E_a we measured P_f at 10, 20, and 30°C. We compared the temperature dependence of the water permeability of three groups of oocytes: water injected and medulla mRNA injected, with or without exposure to cAMP. Fig. 3 shows the Arrhenius plot of one of three experiments. Each symbol represents the P_f of a single oocyte. The E_a values were: 22.0, 10.3, and 6.8 kcal/mol in water-injected, medulla mRNA-injected, and medulla mRNA-injected oocytes

treated with Cl-PheS-cAMP, respectively. We found very similar results in the other two experiments. The high value of E_a in the water-injected oocytes is consistent with water diffusion through the lipid bilayer of the oocyte membrane. In contrast, the much lower value of E_a found in the two groups of oocytes injected with mRNA is consistent with the presence of water channels in the oocyte membrane. In the calculation of the E_a for mRNA-injected oocytes the P_f values include the permeability of both the lipid bilayer and the expressed water channels. Subtracting the P_f value of the water-injected oocytes, thought to correspond to that of the lipid bilayer, the value for E_a obtained will then provide an estimate of the activation energy of the water channel per se. This calculation yields an E_a of 2–4 kcal/mol, a value clearly in the range expected for water channels and similar to that obtained in ADH-sensitive epithelia by others (Kachadorian, Muller, Rudich, and Discala, 1979; Verkman et al., 1988).

Effect of Antisense Oligonucleotide for CHIP28

CHIP28, or a closely related protein, exists in proximal tubules and has been postulated to serve as a water channel (Preston et al., 1992). Therefore, we have tested whether the water permeability expressed after the injection of renal mRNA could be accounted for by the expression of CHIP28. For this purpose we coinjected 40 ng of renal mRNA with 2.27 ng of an antisense oligonucleotide for mouse CHIP28. The proportion of this mixture was chosen to give a 100-fold molar excess of the antisense, assuming that the message for CHIP28 is 1% of the kidney total mRNA. As a preliminary step it was necessary to demonstrate that the antisense inhibited the expression of CHIP28 in oocytes. Fig. 4A shows that the injection into oocytes of a full-length cRNA coding for the human erythrocyte CHIP28 produced a large increase in P_f . The coinjection of this CHIP28-cRNA with the antisense oligonucleotide resulted in an 84% inhibition of the CHIP28-dependent water permeability. P_f was reduced from 120.8 ± 13 to 31.0 ± 3.6 $\mu\text{m/s}$. However, the coinjection of CHIP28 cRNA with a sense oligonucleotide had no effect on the magnitude of the expressed oocyte water permeability ($P_f = 120.3 \pm 10.5$ $\mu\text{m/s}$). Also, the injection of oligonucleotides alone resulted in P_f values not different from those measured in oocytes injected with water (Fig. 4A).

Fig. 4B summarizes the results of experiments in which CHIP28 antisense oligonucleotide was coinjected with renal mRNA. The antisense oligonucleotide reduced P_f by 59% when coinjected with cortex mRNA, by 78% when coinjected with medulla mRNA, and by only 26% when coinjected with papilla mRNA. If we include a correction for the magnitude of the inhibition (84%) of the expression of cRNA CHIP28 by the dose of antisense oligonucleotide used, the percentage inhibition of kidney mRNA is 70 for cortex, 100 for medulla, and 30 for papilla. These results indicate that renal CHIP28 corresponds to most of the water channels expressed upon injection of mRNA from medulla and cortex, but only to a smaller fraction of those appearing after the injection of papilla mRNA. Furthermore, in the papilla there must be a message for a water channel that is different from CHIP28.

We studied the effect of antisense oligonucleotide on the cAMP-stimulated P_f in medulla mRNA-injected oocytes. The results are shown in Fig. 4B. Addition of Cl-PheS-cAMP significantly ($P < 0.005$) increased P_f in oocytes coinjected with the

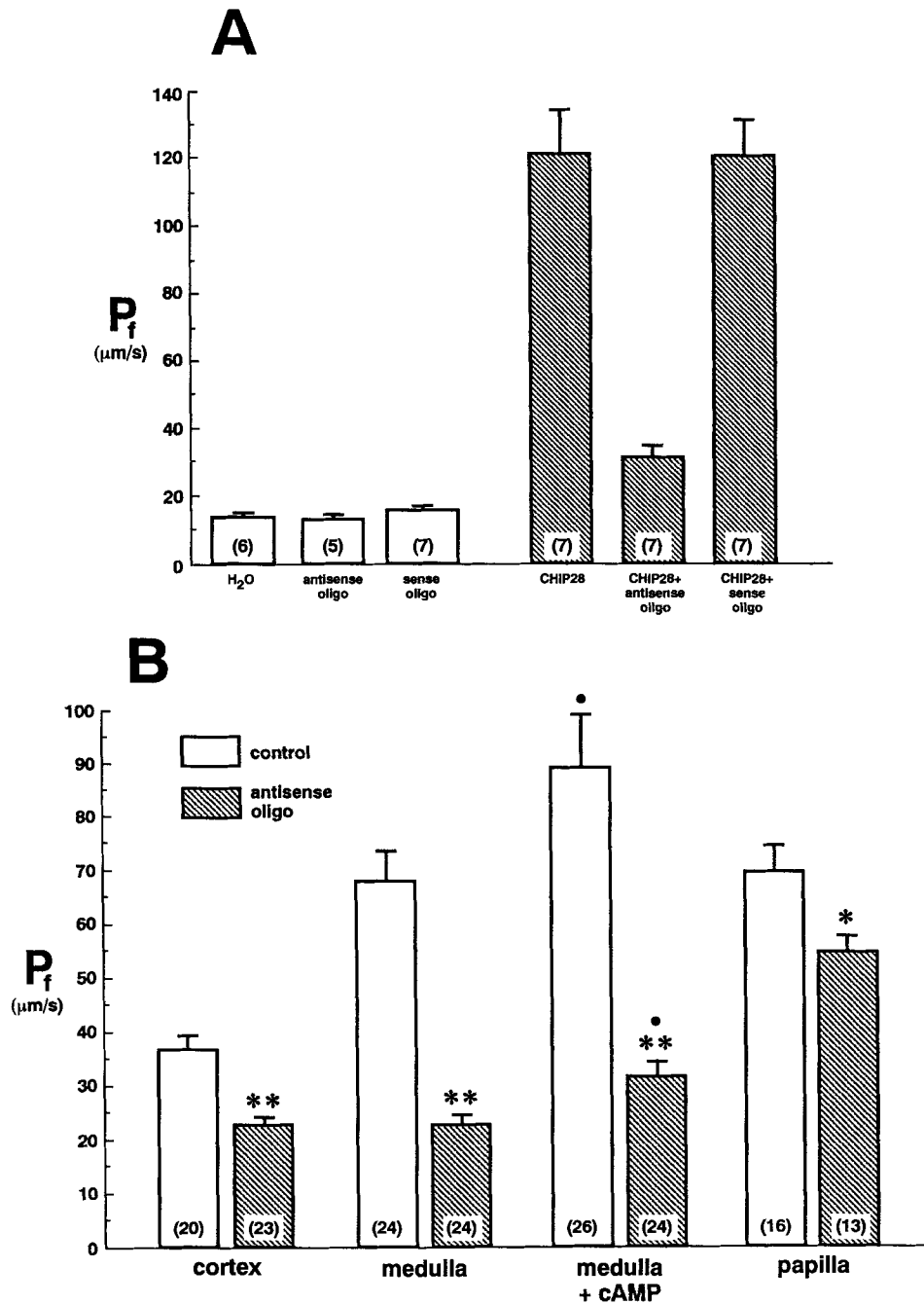


FIGURE 4. Effect of antisense and sense oligonucleotides of CHIP28 on the water permeability of oocytes. Bars represent means \pm SE. The number of oocytes in each group is given in parenthesis. (A) Oocytes were injected with either water (50 nl), antisense oligonucleotide (2.27 ng), sense oligonucleotide (2.27 ng), CHIP28-cRNA (0.2 ng), CHIP28-cRNA (0.2 ng) + antisense oligo (2.27 ng), or CHIP28 (0.2 ng) + sense oligo (2.27 ng). Oocytes injected with

antisense oligonucleotide as well as in oocytes injected only with medulla mRNA. Therefore, the water permeability elicited by cAMP is not altered by the antisense oligonucleotide for CHIP28, suggesting that cAMP activated a channel that is different from CHIP28.

DISCUSSION

In agreement with a preliminary report from this laboratory (Milovanovic et al., 1989) and other subsequent publications (Zhang et al., 1990; Dempster, Van Hoek, and Van Os, 1992), we found expression of water channels after the injection into oocytes of mRNA from either cortex, medulla, or papilla. It is unlikely that the expressed water channels correspond to the insertion into the membrane of endogenous oocyte proteins because the channels are regulated in a manner similar to the regulation of water permeability in the kidney. Thus, the P_f obtained with cortex mRNA was not altered by Cl-PheS-cAMP, whereas that with medulla mRNA was increased. The water permeability expressed after the injection of each of the three renal mRNA preparations had low E_a values (Fig. 3) and was inhibited by pCMBS (Fig. 2A), features that are characteristic for water channels (Finkelstein, 1987).

In the proximal tubule and the descending thin limb of Henle's loop, Agre and his colleagues (Denker et al., 1988; Preston et al., 1992) have identified a protein identical or closely related to erythrocyte CHIP28. They have proposed that this protein is the main water channel in these cells. We expected, therefore, that the message coding for CHIP28 would be present in mRNA prepared from cortex and medulla. To examine whether CHIP28 can account for the water permeability expressed in oocytes injected with renal mRNA we coinjected poly(A⁺) RNA with a CHIP28 antisense oligonucleotide. A marked inhibition of the expression of the human cRNA coding for CHIP28 was observed in oocytes injected with the antisense oligonucleotide (Fig. 4A). Inhibition of equal or larger magnitude should be expected for the expression of rat CHIP28.

Our results indicate that the message for CHIP28 or a highly homologous protein is abundant in cortex and medulla, where its expression is responsible for the major fraction of water permeability expressed in the oocytes. In contrast, the water permeability expressed in oocytes injected with mRNA from papilla is only slightly reduced by CHIP28 antisense oligonucleotide.

The results with cortex mRNA are in agreement with immunolocalization studies demonstrating abundant CHIP28 protein in the proximal tubule, especially in the

either sense or antisense oligonucleotides had the same P_f as water-injected oocytes. Only the antisense oligonucleotide markedly inhibited the expression of CHIP28 water permeability. (B) Oocytes were injected with 40 ng of renal mRNA from either cortex, medulla, or papilla with or without 2.27 ng of the antisense oligonucleotide. Oocytes injected with medulla and medulla + antisense oligonucleotide were subdivided in two subgroups: one from the medulla mRNA-injected subgroup and one from the medulla mRNA + antisense oligonucleotide-injected subgroup were exposed to 10^{-4} M Cl-PheS-cAMP. * and ** indicate values significantly different, $P < 0.05$ and $P < 0.0001$, respectively, from the oocytes not injected with the antisense oligonucleotide. ● indicates values higher ($P < 0.005$) than the corresponding medulla mRNA-injected groups not exposed to Cl-PheS-cAMP.

brush border (Denker et al., 1988; Nielsen et al., 1993). However, we found that ~30% of the expressed water permeability was not accounted for by CHIP28, suggesting the expression of an additional path for water movement after the injection of cortex mRNA. The nature of this additional pathway as well as the cell origin of the message coding for it are unknown. Alternatively, one may propose that the dose of antisense oligonucleotide was insufficient to inhibit the cortex mRNA for CHIP28 to the same extent as with CHIP28 cRNA and medulla mRNA. This possibility is very unlikely because the P_f observed with CHIP28 cRNA was threefold higher than with cortex mRNA and therefore the ratio of antisense oligonucleotide to message for renal CHIP28 should have been more favorable for complete inhibition with cortex than with CHIP28 cRNA. Furthermore, in oocytes injected with papilla mRNA, as expected from the renal distribution of CHIP28 (Denker et al., 1988; Nielsen et al., 1993) and the low abundance of cells from the thin descending limbs of Henle's loop, the contribution of CHIP28 was only ~30%. We speculate that the remaining 70% corresponds to the expression of messages for different water channels which are present in the papillary collecting duct cells. In these cells two populations of water channels have previously been postulated for the apical membrane: one activated by ADH and another that renders the membrane permeable to water in the absence of ADH and is regulated by the animal's state of hydration. In papillary collecting ducts studied *in vitro*, at least half of the P_f increase elicited by water deprivation is accounted for by the ADH independent component (Lankford et al., 1991). The lack of P_f activation by Cl-PheS-cAMP in oocytes injected with papilla mRNA suggests that the most abundant message for water channels in the papilla is that of the ADH independent channel. However, we may have failed to detect the expression of a small number of cAMP activated channels.

In contrast to the findings with cortex and papilla mRNA, in oocytes injected with medulla mRNA and not stimulated with Cl-PheS-cAMP practically all the expressed water permeability corresponded to water flow via CHIP28 channels. In spite of the fact that the kidney papilla was included in the tissue used for the preparation of medulla mRNA, we did not detect in oocytes injected with medulla mRNA the additional (different from CHIP28) water channel that was observed with the injection of pure papilla mRNA. One simple explanation for this result would be that the message for this additional channel was not abundant enough in the medulla mRNA preparations for us to detect its expression in oocytes. It is likely that the water channel activity encoded by mRNA from medulla corresponds to that of the thin descending limb of Henle's loop, a nephron segment known to be highly permeable to water.

Upon stimulation with Cl-PheS-cAMP, an increase in water permeability was only observed in oocytes injected with medulla mRNA. This increase seemed to be due to the expression of water channels because it was abolished by pCMBS (Fig. 2A) and had an E_a of 2–4 kcal/mol (Fig. 3). Two observations indicate that the cAMP-dependent stimulation of P_f resulted from the activation of channels different from CHIP28 protein: (a) Cl-PheS-cAMP did not increase P_f in oocytes injected with CHIP28 cRNA (Fig. 2B), and (b) cAMP-dependent water channel activity was not inhibited by the CHIP28 antisense oligonucleotide (Fig. 4B). On the other hand, this cAMP-dependent water channel seems to differ also from the channel expressed after

injection of papilla mRNA because the latter was not stimulated with Cl-PheS-cAMP. Therefore, these results suggest that the cAMP-stimulated channel expressed in the oocyte may correspond to the water channel inserted in the apical membrane of the collecting tubule cells in response to vasopressin. This channel may be the 53–55-kD protein(s) identified in the apical membrane of ADH-stimulated toad urinary bladder cells (Harris et al., 1991; Calamita, Valenti, Svelto, and Bourguet, 1992).

We cannot rigorously rule out more complicated interpretations of our results. For example, it is plausible that mRNA-injected oocytes expressed a kidney-specific protein that in turn induced the expression of endogenous oocyte water channels. However, the good correlation between the expressed water permeability in mRNA-injected oocytes and the type of water channels known to exist in the corresponding sections of the kidney from which the mRNA was obtained makes the above possibility rather unlikely.

The mechanism of the activation of water channels by cAMP in oocytes injected with medulla mRNA is unknown, but two possibilities may be considered. First, the channels are present in the cell membrane in a closed state and are opened by a cAMP-dependent process. The second possibility is that the channels are constantly permeable to water but are stored in a cytoplasmic compartment and inserted into the oocyte membrane by a cAMP-activated mechanism. This latter possibility resembles the events that take place in the collecting tubule after exposure to vasopressin. In both cases the cAMP activation would probably need the presence of intracellular components that would link the rise in cAMP concentration with the increase in P_f . We do not know whether these additional components are present in the oocytes before the injection of medulla mRNA or are only expressed as a result of the injection of mRNA.

In summary, our results are consistent with the existence of three different water channels in kidney tissue: these channels would include CHIP28, a cAMP-activated channel, and a papillary channel that is independent of the action of cAMP and presumably independent of vasopressin.

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