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Synergistic Effects of Cystic Fibrosis Transmembrane Conductance Regulator and Aquaporin-9 in the Rat Epididymis[1]

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ABSTRACT

The cystic fibrosis transmembrane conductance regulator (CFTR) and aquaporin-9 (AQP-9) are present in the luminal membrane of the epididymis, where they play an important role in formation of the epididymal fluid. Evidence is accumulating that CFTR regulates other membrane transport proteins besides functioning as a cAMP-activated chloride channel. We have explored the possible interaction between epididymal CFTR and AQP-9 by cloning them from the rat epididymis and expressing them in *Xenopus* oocytes. The effects of the expressed proteins on oocyte water permeability were studied by immersing oocytes in a hypo-osmotic solution, and the ensuing water flow was measured using a gravimetric method. The results show that AQP-9 alone caused an increase in oocyte water permeability, which could be further potentiated by CFTR. This potentiation was markedly reduced by phloretin and lonidamine (inhibitors of AQP-9 and CFTR, respectively). The regulation of water permeability by CFTR was also demonstrated in intact rat epididymis luminally perfused with a hypo-osmotic solution. Osmotic water reabsorption across the epididymal tubule was reduced by phloretin and lonidamine. Elevation of intracellular cAMP with 3-isobutyl-1-methylxanthine increased osmotic water permeability, whereas inhibiting protein kinase A with H-89 (*N*-(2-[*p*-bromocinnamylamino]ethyl)-5-isoquinoline sulfonamide hydrochloride) reduced it. These results are consistent with a role for CFTR in controlling water permeability in the epididymis in vivo. We conclude that this additional role of CFTR in controlling water permeability may have an impact on the genetic disease cystic fibrosis, in which men with a mutated *CFTR* gene have abnormal epididymis and infertility.

aquaporin-9, cystic fibrosis transmembrane conductance regulator, epididymis

INTRODUCTION

The epididymal epithelial cells transport electrolytes and water to create an intraluminal fluid conducive to sperm maturation and storage. These transport processes are under tight control by nerves and hormones. Various neurohumoral agents influence fluid secretion via G-protein-coupled receptors linked to various second-message cascades culminating in the activation of ion channels. Of particular importance is the apically placed cystic fibrosis transmembrane conductance regulator (CFTR), which functions as a cAMP-activated chloride channel [1, 2]. This channel provides an exit pathway for secondary active chloride transport from blood to lumen, with sodium following passively. The resulting accumulation of NaCl in the lumen generates an osmotic gradient for water secretion via the water channels, aquaporins. One aquaporin isoform, aquaporin-9 (AQP-9), has been shown to be expressed by the rat epididymis [3]. Both CFTR and AQP-9 are present in the luminal membrane of the principal cells of the epididymis but not in the intercalated cells. The CFTR-Cl^- channel serves as a conductive pathway for anions, whereas AQP-9 functions as a water channel. Both proteins are therefore essential to formation of the epididymal fluid. Evidence from other tissues indicates that CFTR serves as a regulator of other membrane transport proteins besides functioning as a cAMP-activated Cl^- channel (for review, see [4]). In the present study, we investigated whether CFTR interacts with AQP-9 in controlling water permeability in the epididymis. Such an interaction may have an impact on the genetic disease cystic fibrosis, in which men with a mutated *CFTR* gene are known to have abnormal epididymal morphology and function.

MATERIALS AND METHODS

RNA Isolation and Cloning of CFTR and AQP-9

All experiments on animals were conducted in accordance with the guidelines on the use of laboratory animals laid down by the Animal Research Ethics Committee of the Chinese University of Hong Kong. Epithelial cells of the rat cauda epididymides were prepared as described previously [5, 6]. Total RNA from rat epididymal epithelial cells was isolated using TRIzol reagent (Gibco BRL, Grand Island, NY). Two micrograms of total RNA were used for first-strand cDNA synthesis using oligo$(dT)_{18}$ primer and Superscript II RNase H^- Reverse Transcriptase (SuperScript Preamplification System; Gibco BRL). The resulting first-strand cDNA was directly used for polymerase chain reaction (PCR) amplification.

Two sets of primers were designed to amplify the full-length CFTR and AQP-9 cDNAs according to the published sequences [7, 8]. The first primer pair used for amplifying the CFTR was 5′-CACGGTACCATCATGCAGAAGTCGCCTCTG-3′ (sense; corresponding to nucleotides −12 to 18) and 5′-AGCCTCGAGCACTAGAGCCGGGTCTCTTGC-3′ (antisense; corresponding to nucleotides 4419 to 4448), which generated a 4.4-kilobase, full-length CFTR cDNA. The second primer pair used for amplifying full-length AQP-9 was 5′-TAGTGAAAGCAGGGAACCGAGC-3′ (sense; corresponding to nucleotides −66 to −45) and 5′-AGAACTGGATGAACGGTAACTG-3′ (antisense; corresponding to nucleotides 906 to 927), which generated a 993-base pair AQP-9 cDNA. The PCRs were carried out by combining the following reagents in a final volume of 50 μl: 1× PCR buffer, 1.5 mM $MgCl_2$, 5 mM deoxynucleotide triphosphate, 10 μM primer pair, 1 U of *Taq* DNA polymerase, and 2 μl of cDNA template. These PCR mixtures were subjected to 25–28 cycles at 95°C for 1 min, 55°C for 1 min, and 72°C for 5 min (for CFTR) or 1 min (for AQP-9). The PCR products were resolved on a 1.2% agarose gel and visualized under ultraviolet light followed by ethidium bromide staining. PCR products with expected size of full-length fragment were gel purified from the agarose gel. The purified PCR products were cloned into pGEM-T cloning vector (Promega, Madison, WI) according to the manual supplied.

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TABLE 1. Sequence of primers used for the generation of truncated CFTR fragments.

Domain amplified	Primer sequence[a]	Corresponding size (kilobases)
M595-C (C-terminal half of CFTR including R domain but no NBD1)	F: 5′-TTTGAAAAGTTGTGTCTGT-3′ R: 5′-AGCCTCGAGCACTAGAGC-3′	2.7
E402X (N-terminal half of CFTR but no NBD1)	F: 5′-AGACATCATGCAGAAGTCGCCT-3′ R: 5′-CTCCCAAAATGCTGTTACATT-3′	1.2
W401M-D651X (NBD1 only)	F: 5′-ACAGCAACCTGGAGGAGGGATTTCAG-3′ R: 5′-CTCGAGCATAAAAGTATCATACCCCAT-3′	0.8

[a] F, forward; R, reverse.

Generation of CFTR Truncated Fragments

Different CFTR truncated fragments were generated by PCR as described previously [9, 10]. In brief, the truncated forms of CFTR, M595-C (C-terminal half of CFTR including R domain but lacking NBD1), E402X (N-terminal half of CFTR but lacking NBD1), and W401M-D651X (NBD1 only), were PCR amplified by specific primers (Table 1) and cloned into pGEM-T cloning vectors (Promega). The authenticity of the truncated fragments was checked by DNA sequencing (ABI PRISM 310 Automated DNA Sequencer; Applied Biosystems, Foster City, CA).

Preparation of cRNA and Xenopus Oocytes for Microinjection

For the synthesis of cRNAs, in vitro transcription was preformed. Plasmids containing the full-length AQP-9, CFTR, and truncated forms of CFTR were linearized by restriction enzymes. The corresponding cRNAs were synthesized using T7 promoter with the respective polymerase and 5′-capped (mCAP mRNA capping kit; Stratagene, La Jolla, CA).

The procedures for isolation and microinjection of oocytes have been described previously [11]. Briefly, after the oocytes were isolated from the adult *Xenopus* female (Nasco, Fort Atkinson, WI), the oocytes were defolliculated by 1-h digestion with collagenase (type IAp; Sigma, St. Louis, MO). Oocytes were then washed several times and stored in ND96 buffer: 96 mmol/L of NaCl, 2 mmol/L of KCl, 1.8 mmol/L of $CaCl_2$, 1 mmol/L of $MgCl_2$, 5 mmol/L of Hepes, 2.5 mmol/L of sodium pyruvate, pH 7.5, supplemented with 0.5 mmol/L of theophylline and 5 mg/L of gentamicin at 18°C. Oocytes of identical batches were each injected with 10–50 ng of cRNA (CFTR, AQP-9, or CFTR + AQP-9 coinjected) and dissolved in 50 nl of water. In some experiments, the truncated form of CFTR was coinjected with AQP-9 cRNA into oocytes. The water-injected oocytes served as controls. Oocytes were preincubated with phloretin (100 μM) or lonidamine (300 μM) for 15 min before immersion in hypo-osmotic solution. The scheme of cloning and expression of epididymal CFTR and AQP-9 in *Xenopus* oocytes is illustrated in Figure 1.

Osmotic Water Permeability Measurement

The osmotic water permeability coefficient (P_f) was calculated by the rate of hypotonic volume increase measured by a gravimetric method at 22°C as described previously [12]. In brief, fresh mass of 10 pooled (control or cRNA-injected) oocytes was measured with an electronic balance (Mettler AJ100, Greifensee, Switzerland) three to five times, and the mean value was then taken. Normotonic medium (220 ± 4 mOsmol/L [± SEM]) was prepared from ND96 buffer by substitution of 120 mmol/L of mannitol for 60 mmol/L of NaCl. Hypotonicity was induced by omitting the mannitol from the normotonic medium. The P_f value was calculated according to the method of Echevarria et al. [13]:

$$P_f = dV/dt \cdot [1/S \cdot \Delta\pi]$$

where dV/dt is the weight change 1 min after exposure to hypotonic medium, S is the surface area of the oocyte, and $\Delta\pi$ is the osmolarity gradient at time zero. 3-Isobutyl-1-methylxanthine (IBMX; 500 μM) was used to activate the expressed CFTR on the oocytes during P_f measurement. Results are expressed as the mean ± SEM. Statistical analysis of the data was performed by unpaired Student *t*-test.

Osmotic Water Reabsorption in Intact Rat Epididymis In Vivo

Osmotic water flow was studied in intact epididymis of mature rats in vivo using the luminal perfusion technique. Adult male Sprague-Dawley rats weighing 350–400 g (age, 80 days) were used in this study. All experiments on animals were conducted in accordance with the guidelines on the use of laboratory animals laid down by the Animal Research Ethics Committee of the Chinese University of Hong Kong. Details of the procedure for the cannulation and microperfusion of the cauda epididymal duct have been described previously [14, 15]. In brief, net water transport across the luminally perfused rat epididymis was measured using inulin as an intraluminal volume marker. [^{3}H]Inulin (125 nCi/ml; specific activity, 122 μCi/mg; Amersham, Buckinghamshire, U.K.) was added to the

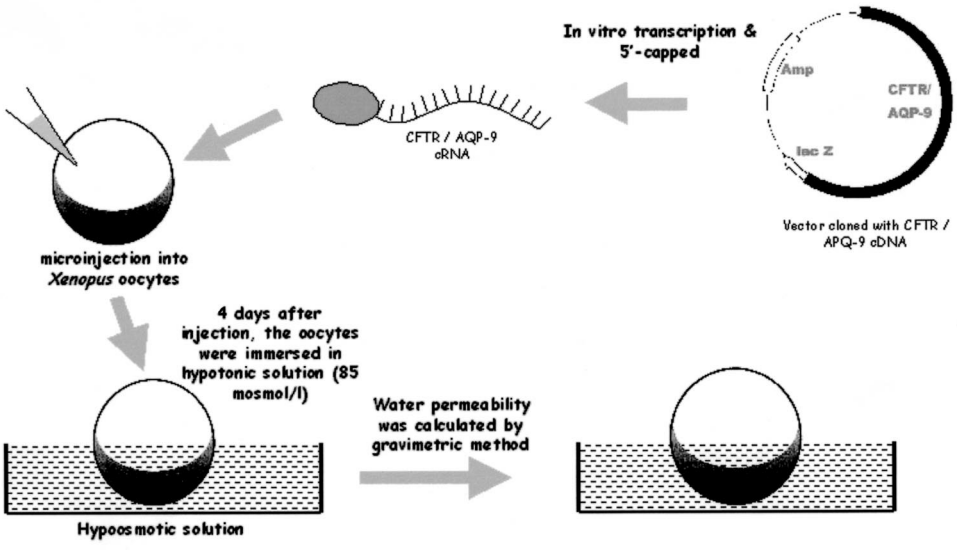

FIG. 1. Schematic diagram showing the cloning and in vitro transcription of CFTR and AQP-9. Full-length CFTR and AQP-9 cDNA obtained by reverse transcription-PCR were cloned into expression vectors. After in vitro transcription using T7 RNA polymerase, CFTR and AQP-9 cRNAs were injected into *Xenopus* oocytes for measurement of osmotic water permeability (P_f).

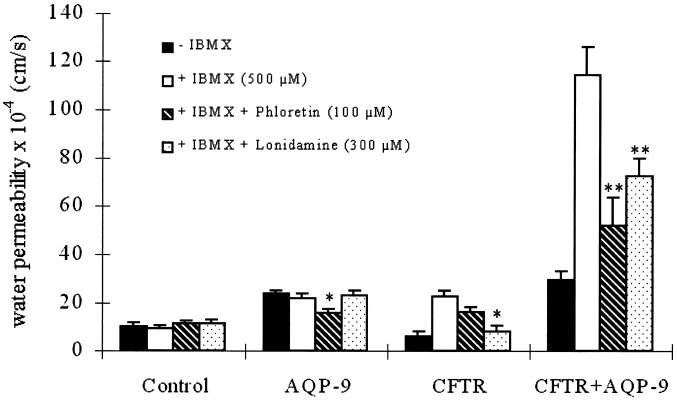

FIG. 2. Water permeability (P_f) of *Xenopus* oocytes expressed with CFTR and AQP-9 cRNAs. Osmotic water permeability of oocytes immersed in hypotonic solution (85 mOsmol/L) was measured 4 days after injection of cRNAs. Injected alone, CFTR or AQP-9 cRNA increased water permeability. The effect of CFTR required the presence of IBMX. When coinjected into the oocytes, CFTR in the presence of IBMX potentiated the effect of AQP-9 in inducing water permeability. The effects of phloretin (100 μM) and lonidamine (300 μM) are also shown. Asterisks indicated significant difference from oocytes stimulated with IBMX within the same experimental group (*$P < 0.05$, **$P < 0.01$).

perfusing solution, which was hypo-osmotic with respect to blood plasma. The solution contained 25 mmol/L of NaCl and 5 mmol/L of Hepes (pH 7.4). The calculated osmolarity was 55 mOsmol/L. In some experiments, isotonic solution was used to perfuse the epididymis. This solution contained 117 mmol/L of NaCl, 4.7 mmol/L of KCl, 1.2 mmol/L of $MgCl_2$, 1.2 mmol/L of KH_2PO_4, 24.8 mmol/L of $NaHCO_3$, 2.56 mmol/L of $CaCl_2$, and 11.1 mmol/L of glucose. When gassed with 5% CO_2, it has a pH of 7.4. The calculated osmolarity was 290 mOsmol/L. Perfusion was carried out at a rate of 1.5 μl/min. Histological sections were prepared to check the integrity of the epithelium. It was found that perfusion with a hypo-osmotic solution for a few hours did not cause apparent damage to the epithelium. The activities of radioactive inulin in both the perfusate and the original perfusion solution were measured over intervals of 30 min, and their ratio was used to calculate the net water flux, which was expressed in terms of nl cm^{-1} min^{-1} [14]. In most cases, the rate of net water transport in a luminally perfused epididymis was followed over a period of more than 200 min, during which time drugs were added to the perfusion fluid as described.

Chemicals and Drugs

β-(4-Hydroxyphenyl)-2,4,6-trihydroxypropiophenone (phloretin), *N*-(2-[*p*-bromocinnamylamino]ethyl)-5-isoquinoline sulfonamide hydrochloride (H-89), and IBMX were purchased from Sigma. Lonidamine was a gift from Prof. B. Silvestrini (University of Rome, Rome, Italy) and Dr. Yan Cheng (Population Council, New York, NY).

RESULTS

Activation of AQP-9 by CFTR

Oocytes injected with water (50 nl) showed a small increase in weight when immersed in hypo-osmotic solution, presumably caused by intrinsic water permeability of the oocyte membrane. In oocytes injected with AQP-9 cRNA, water permeability was increased compared to that of the water-injected control. This increase was not dependent on the presence of an elevated level of intracellular cAMP, but it was significantly reduced by phloretin (Fig. 2). This is probably caused by insertion of the water-channel AQP-9 into the oocyte membrane, hence allowing an increase in water permeability to occur. Oocytes injected with CFTR cRNA alone also caused an increase in water permeability compared to that of the water-injected control, but the increase required an elevated level of intracellular cAMP. This effect of CFTR was significantly reduced by lonidamine (Fig. 2). The largest increase in water permeability was observed when CFTR cRNA and AQP-9 cRNA were coinjected into the oocytes in the presence of IBMX. This increase in water permeability, which exceeded the sum of the increases caused by CFTR and AQP-9 alone, was markedly attenuated by preincubating the oocytes with phloretin (100 μM). Lonidamine (300 μM) also significantly reduced the increase of water permeability brought about by coexpression of the two proteins (Fig. 2).

Effects of Truncated Versions of CFTR

Three truncated forms of CFTR were prepared from PCR fragments. The ability of these constructs in potentiating AQP-9 was compared to that of wild-type CFTR. (Fig. 3). Neither M595-C (N-terminal half of CFTR lacking

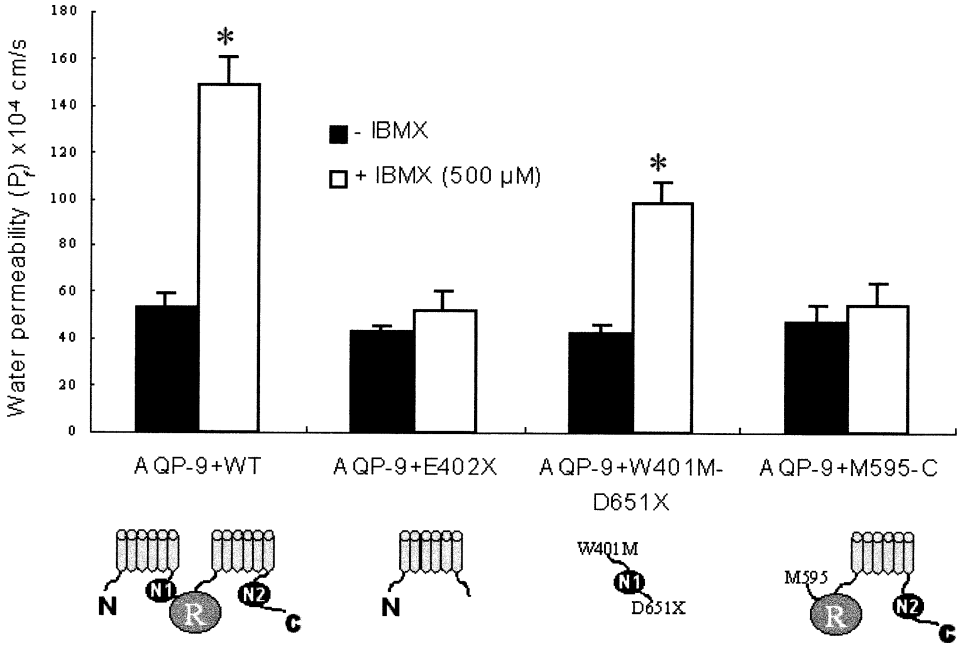

FIG. 3. Effects of coexpression of wild-type (WT) CFTR and truncated CFTRs with AQP-9 on osmotic water permeability in oocytes in the presence or absence of IBMX (see text for explanation). Asterisks indicated significant difference from respective controls without IBMX (*$P < 0.01$).

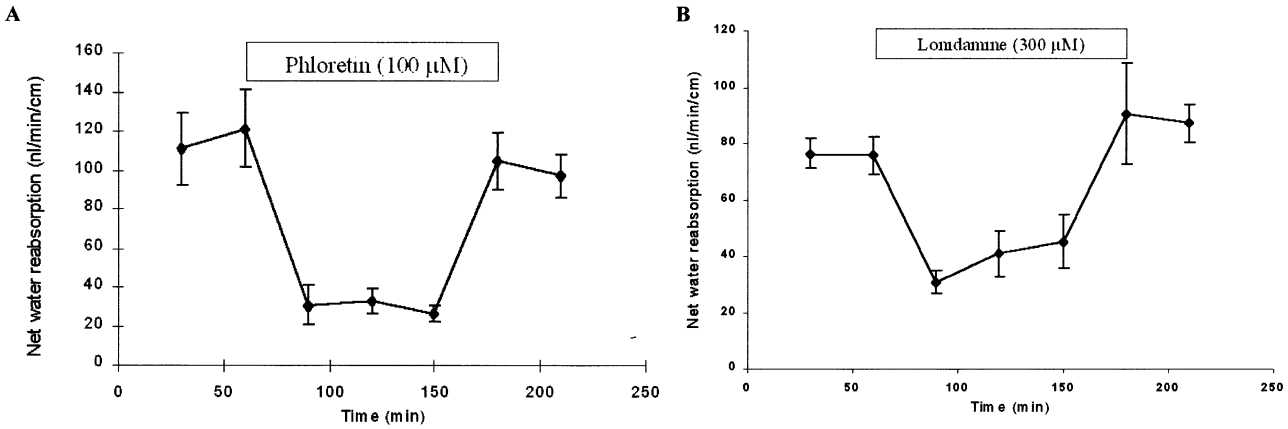

FIG. 4. Osmotic water reabsorption in intact rat epididymis. **A**) Rat cauda epididymides were luminally perfused with hypo-osmotic solution (55 mOsmol/L), and net water reabsorption was measured using [3H]inulin as a fluid volume marker. Phloretin (100 μM; open bar) added to the perfusate markedly and reversibly inhibited osmotic water flow. Each point shows the mean + SEM of five experiments. **B**) Addition of lonidamine (300 μM), an inhibitor of CFTR, reduced osmotic water permeability of the perfused rat cauda epididymides. This inhibitory effect can be reversed on removal of lonidamine from the perfusate. Each point shows the mean + SEM of five experiments.

NBD1) nor E402X (C-terminal half of CFTR including R domain but lacking NBD1) potentiated AQP-9. However, W40M-D651X (a fragment containing NBD1 only) preserved some of the activities of wild-type CFTR in potentiating AQP-9-induced water permeability in the oocytes (Fig. 3).

Osmotic Water Reabsorption in Intact Rat Epididymis In Vivo

The interaction between CFTR and AQP-9 in regulating osmotic water permeability was also studied in intact cauda epididymides of anesthetized rats in vivo. When luminally perfused with a hypo-osmotic solution (55 mOsmol/L), the epididymal duct reabsorbed water at a rate of approximately 100 nl min^{-1} cm^{-1}. Addition of phloretin (100 μM), an inhibitor of AQP-9, to the perfusing fluid markedly reduced the reabsorption rate (Fig. 4A). This effect was readily reversible on removal of phloretin from the perfusing solution. Addition of lonidamine (300 μM), an inhibitor of CFTR [16, 17], to the perfusing solution also reduced water reabsorption. The effect was reversible on removal of lonidamine from the perfusate (Fig. 4B).

The effect of intracellular cAMP on osmotic water permeability of intact epididymal tubules was also studied. Water reabsorption was first measured in epididymal tubules luminally perfused with isotonic Krebs solution. The reabsorption rate was approximately 18 nl min^{-1} cm^{-1}. The perfusion fluid was then shifted to a hypo-osmotic solution (55 mOsmol/L); thereafter, the reabsorption rate increased to approximately 100 nl min^{-1} cm^{-1} after 50 min (Fig. 5A). When the reabsorption rate had become steady, H-89 (20 μM), an inhibitor of protein kinase A (PKA), added luminally reduced the reabsorption rate to the level seen with isotonic solution.

In another set of experiments, epididymal tubules were perfused luminally with a hypo-osmotic solution (55 mOsmol/L) to attain a reabsorption rate of approximately 100 nl min^{-1} cm^{-1}. Then, IBMX (500 μM) was added to the perfusate to boost intracellular cAMP. The water reabsorption rate was further increased to approximately 140 nl min^{-1} cm^{-1}. At the steady state, lonidamine (300 μM), an inhibitor of CFTR, added luminally markedly reduced the reabsorption rate.

DISCUSSION

Both CFTR [18, 19] and AQP-9 [3] have been shown to be expressed by the rat and human epididymis. They are

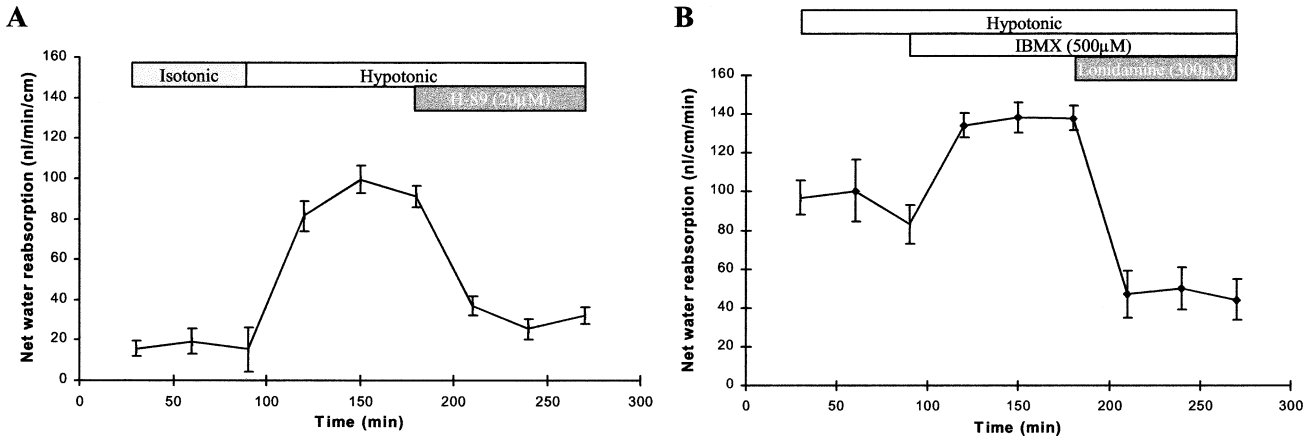

FIG. 5. Involvement of PKA and cAMP pathway in hypotonic water reabsorption. **A**) Net water reabsorption in intact rat epididymis was increased when perfusate was shifted from an isotonic to a hypo-osmotic solution. Water reabsorption was significantly reduced by H-89 (20 μM), a PKA inhibitor, added luminally. **B**) Hypo-osmotic water reabsorption was increased significantly when IBMX (500 μM), a phosphodiesterase inhibitor, was added to the perfusate. Subsequent addition of lonidamine (300 μM) reduced the water reabsorption rate. Each point shows the mean + SEM of five experiments.

localized on the luminal membrane of the principal cells, where they play a role in transporting electrolytes and water and are therefore important in formation of the epididymal fluid. Evidence in other epithelial tissues indicates that CFTR, besides functioning as a cAMP-activated chloride channel, also acts as a regulator of other membrane transport proteins. Among them are the epithelial Na^+ channels [10] and the outwardly rectifying Cl^- channels [20]. Recent evidence also indicates that CFTR interacts with one of the water channels, AQP-3, in human airway epithelial cells [21]. It is claimed that such interaction may be of clinical relevance to cystic fibrosis, in which mutation of the CFTR gene has led to abnormal pulmonary functions.

We have expressed rat CFTR and AQP-9 cloned from the rat epididymis in *Xenopus* oocytes to study the interaction between the two membrane proteins in conferring water permeability. The results show a significant increase in water permeability when both proteins were expressed, and the increase was greater than the sum of the water permeability caused by the expression of either protein alone. This finding is consistent with a synergistic effect of the two proteins in conferring water permeability in the oocytes. The effect was markedly reduced by phloretin, a drug known to block some isoforms of aquaporin, including AQP-9 [8, 22], and by lonidamine, a drug known to block CFTR [16]. We have pinpointed the domain within the CFTR molecule that is responsible for the interaction by using truncated versions of CFTR. We found that the first nucleotide-binding domain (NBD1) is essential for activity, because the N-terminal half and the C-terminal half of the CFTR devoid of NBD1 are incapable of potentiating AQP-9. In contrast, the CFTR fragment containing the NBD1 only preserves some degree of activity. In a separate study [18], we have characterized the molecular mechanism of the block of CFTR-Cl^- channels by lonidamine and found that lonidamine caused a flickery block of the channel and reduced channel open time at low concentrations (<50 μM) but affected channel gating properties by reducing open channel probability at higher concentrations (>50 μM). It is generally held that flickery (i.e., open channel) block of ion channels is usually caused by interaction of the channel blocker with the pore region of the channel whereas changes in gating properties are caused by interaction with the regulatory sites (e.g., the nucleotide-binding domains in CFTR). The patch-clamp work therefore suggests that at high concentrations, lonidamine binds to the nucleotide-binding domains of CFTR. Our present finding that lonidamine (300 μM)-mediated attenuation of the potentiating effect of CFTR on AQP-9 requires the presence of NBD1 is consistent with the findings suggested by the patch-clamp studies.

The role of CFTR in regulating water permeability is also demonstrated in intact epididymis in vivo. When luminally perfused with a hypo-osmotic solution, the epididymis underwent a net water reabsorption (Fig. 4A). Histological examination of the tubules showed the epithelium was intact after perfusion with a hypo-osmotic solution (results not shown). Water reabsorption under an osmotic gradient was markedly reduced by phloretin (100 μM) added to the perfusing solution. A similar degree of inhibition was also seen when lonidamine (300 μM) was added to the luminal perfusate (Fig. 4B). These results indicate that osmotic water flow in the epididymis is mediated through a phloretin-sensitive aquaporin (e.g., AQP-9) and that CFTR may also be involved in the regulation of osmotic water flow. To our knowledge, no evidence to date suggests that CFTR functions as a water channel per se, and the likely explanation of the results is that CFTR activation is required for AQP-9 to increase water permeability. In the epididymis, CFTR is constitutively expressed. In the presence of a basal level of intracellular cAMP, it mediates basal chloride secretion, which can be measured as short-circuit current [6]. It is plausible that under basal condition, CFTR also activates AQP-9, which is also known to be constitutively expressed, to maintain basal water permeability. Inhibiting CFTR by lonidamine therefore inhibits osmotic water flow by removing the activating effect of CFTR on AQP-9 (Fig. 4B).

The role of intracellular cAMP in controlling osmotic water permeability can be seen in Figure 5. Elevating intracellular cAMP with IBMX is seen to increase osmotic water permeability. This increase could be reversed by lonidamine, an inhibitor of CFTR (Fig. 5B). Furthermore, inhibiting PKA with H-89 also reduced basal water reabsorption driven by an osmotic gradient (Fig. 5A). These results can be interpreted as meaning that in the basal state, the basal CFTR activity maintained by the basal cAMP level (via PKA-dependent phosphorylation) is required to confer water permeability on the epididymal epithelium. This interaction between CFTR and AQP-9 is disrupted by the CFTR-inhibitor lonidamine (Fig. 5B).

This potentiating effect of CFTR on AQP-9 may be important in the efferent duct, which has been shown to express CFTR and AQP-9. Microperfusion experiments have indicated that more than 90% of the testicular fluid is reabsorbed there [23]. In the efferent duct, the primary role of CFTR may be to regulate AQP-9 to achieve a high hydraulic conductance; its role as a cAMP-activated chloride channel is probably of secondary importance. However, in the cauda epididymides, where most of the testicular fluid would have been reabsorbed, regulated secretion of electrolytes and fluid is imperative to prevent luminal dehydration. Under the circumstances, the roles of CFTR as a cAMP-activated Cl^- channel and as a regulator of AQP-9 would be of equal importance.

In conclusion, the present study has demonstrated that rat epididymis CFTR and AQP-9 expressed in *Xenopus* oocytes interact to regulate water permeability of the oocytes. This interaction likely takes place in intact epididymis in vivo. The finding that CFTR regulates water permeability in addition to its role as a chloride channel has raised the possibility that abnormal epididymal development in cystic fibrosis may be caused by abnormal water transport in addition to defective chloride transport in embryonic tissues.

REFERENCES

1. Wong PYD. CFTR gene and male fertility. Mol Human Reprod 1998; 4:107–110.
2. Wong PYD, Gong XD, Leung GPH, Cheuk BLY. Formation of the epididymal fluid microenvironment. In: Robaire R, Hinton B (eds.), The Epididymis: From Molecules to Clinical Practice—A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens. New York: Kluwer Plenum Press; 2002: 119–130.
3. Pastor-Soler N, Bagnis C, Sabolic I, Tyszkowski R, McKee M, Van Hoek A, Breton S, Brown D. Aquaporin 9 expression along the male reproductive tract. Biol Reprod 2001; 65:384–393.
4. Kunzelmann K. CFTR: Interacting with everything? News Physiol Sci 2001; 16:167–170.
5. Cuthbert AW, Wong PYD. Electrogenic anion secretion in cultured rat epididymal epithelium. J Physiol 1986; 378:335–345.
6. Wong PYD. Mechanisms of adrenergic stimulation of anion secretion in cultured rat epididymal epithelium. Am J Physiol 1988; 254:F121–F133.
7. Fiedler MA, Nemcz ZK, Shull GE. Cloning and sequence analysis of

rat cystic fibrosis transmembrane conductance regulator. Am J Physiol 1992; 262:L779–L784.
8. Tsukaguchi H, Shayakul C, Berger UV, Mackenzie B, Devidas S, Guggino WB, van Hoek AN, Hediger MA. Molecular characterization of a broad selectivity neutral solute channel. J Biol Chem 1998; 273: 24737–24743.
9. Kunzelmann K, Kiser G, Schreiber R, Riordan JR. Inhibition of epithelial Na^+ currents by intracellular domains of the cystic fibrosis transmembrane conductance regulator. FEBS Lett 1997; 400:341–344.
10. Schreiber R, Hopf A, Mall M, Greger R, Kunzelmann K. The first-nucleotide binding domain of the cystic-fibrosis transmembrane conductance regulator is important for inhibition of the epithelial Na^+ channel. Proc Natl Acad Sci U S A 1999; 96:5310–5315.
11. Lacal JC. Microinjection of *Xenopus laevis* oocytes: a model system. In: Lacal JC, Perona R, Feramisco J (eds.), Microinjection. Boston: Birkhauser Verlag; 1999: 134–145.
12. Schreiber R, Greger R, Nitschke R, Kunzelmann K. Cystic fibrosis transmembrane conductance regulator activates water conductance in *Xenopus* oocytes. Pflugers Arch 1997; 434:841–847.
13. Echevarria M, Frindt G, Preston GM, Milovanovic S, Agre P, Fischbarg J, Windhager EE. Expression of multiple water channel activities in *Xenopus* oocytes injected with mRNA from rat kidney. J Gen Physiol 1993; 101:827–841.
14. Wong PYD, Yeung CH. Absorptive and secretory functions of the perfused rat cauda epididymides. J Physiol 1978; 275:13–26.
15. Au CL, Wong PYD. Luminal acidification by the perfused rat cauda epididymides. J Physiol 1980; 309:419–427.
16. Gong XD, Wong YL, Leung GPH, Cheng CY, Silvestrini B, Wong PYD. Lonidamine and analogue AF2785 block the cyclic adenosine 3′,5′-monophosphate-activated chloride current and chloride secretion in the rat epididymis. Biol Reprod 2000; 63:833–838.
17. Leung GPH, Gong XD, Cheung KH, Cheng-Chew SB, Wong PYD. Expression of cystic fibrosis transmembrane conductance regulator in rat efferent duct epithelium. Biol Reprod 2000; 64:1509–1515.
18. Gong XD, Linsdell P, Cheung KH, Leung GPH, Wong PYD. Indazole inhibition of cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channels in rat epididymal epithelial cells. Biol Reprod 2002; 67:1888–1896.
19. Wong PYD, Huang SJ, Leung AYH, Fu WO, Chung YW, Zhou TS, Yip WWK, Chan WKL. Physiology and pathophysiology of electrolyte transport in the epididymis. In: Nieschlag E (eds.), Spermatogenesis-Fertilization-Contraception: Molecular, Cellular and Endocrine Events in Male Reproduction. Heidelberg, Germany: Springer-Verlag: Schering Foundation Series; 1992: 319–334.
20. Schwiebert EM, Egan ME, Hwang T-H, Fulmer SB, Allen SS, Cutting GR, Guggino WB. CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. Cell 1995; 81:1063–1073.
21. Schreiber R, Nitschke R, Greger R, Kunzelmann K. The cystic fibrosis transmembrane conductance regulator activates aquaporin 3 in airway epithelial cells. J Biol Chem 1999; 274:11811–11816.
22. Ford P, Merot J, Jawerbaum A, Gimeno MA, Capurro C, Parisi M. Water permeability in rat oocytes at different maturity stages: aquaporin-9 expression. J Membr Biol 2000; 176:151–158.
23. Clulow J, Jones RC, Hansen LA. Micropuncture and cannulation studies of fluid composition and transport in the ductuli efferentes testis of the rat: comparisons with the homologous metanephric proximal tubule. Exp Physiol 1994; 79:915–928.