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# Reconstitution of a Regulated Transepithelial Water Pathway in Cells Transfected with AQP2 and an AQP1/AQP2 Hybrid Containing the AQP2-C Terminus

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**Abstract.** Transepithelial water permeability was measured in LLC-PK1 cells stably transfected with aquaporins (AQPs): AQP1, AQP2, and a chimera of AQP1 and AQP2 containing 41 amino acids of the C-terminus of AQP2. Transepithelial water fluxes (Jw) were not previously reported in cells transfected with aquaporins. Jw were now recorded each minute using a specially developed experimental device. A significant increase in Posm after forskolin (FK) plus vasopressin (VP) was found in AQP2 transfected cells ($39.9 \pm 8.2$ *vs.* $12.5 \pm 3.3$ $cm \cdot sec^{-1} \cdot 10^{-3}$), but not in cells transfected with AQP1 ($15.3 \pm 3.6$ *vs.* $13.4 \pm 3.6$ $cm \cdot sec^{-1} \cdot 10^{-3}$). In the case of the AQP1/2 cells (chimera) the FK plus VP induced Posm was smaller than in AQP2 cells but significantly higher than in mock cells at rest ($18.1 \pm 4.8$ *vs.* $6.7 \pm 1.0$ $cm \cdot sec^{-1} \cdot 10^{-3}$). The increases in Posm values were not paralleled by increases in $^{14}C$-Mannitol permeability. $HgCl_2$ inhibited the hydrosmotic response to FK plus VP in AQP2 transfected epithelia. Results were comparable to those observed, in parallel experiments, in a native ADH-sensitive water channel containing epithelial barrier (the toad urinary bladder). Electron microscopy showed confluent LLC-PK1 cells with microvilli at the mucosal border. The presence of spherical or elongated intracellular vacuoles was observed in AQP2 transfected cells, specially after FK plus VP stimulus and under an osmotic gradient. These results demonstrate regulated transepithelial water permeability in epithelial cells transfected with AQP2.

**Key words:** Water flux — Forskolin — Vasopressin — LLC-PK1 cells — Mercury chloride — Toad urinary bladder

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## Introduction

The aquaporins (AQPs) are a family of homologous intrinsic proteins that are responsible for the high water permeability observed in different cell membranes [13, 25]. AQP1 was the first to be identified as a water channel in the erythrocyte [21] and in tubule epithelium and vascular endothelium in the rat kidney [28, 14]. It is a constitutively expressed protein. In contrast, AQP2 is a regulated protein: It shows significant intracellular accumulation inside vesicles and, after the appropriate physiological stimulus, there is an exocytic fusion with the plasma membrane [12]. AQP2 is expressed in the kidney collecting duct principal cells [5, 4, 15] and its transfer to the apical membrane is controlled by antidiuretic hormone (ADH) [24, 16, 7].

The stable transfection of AQP1 and AQP2 in LLC-PK1 cells has been previously reported [11]. LLC-PK1 is a polarized epithelial cell line derived from pig kidney expressing the vasopressin V2 receptor and shows an increase in intracellular cyclic-AMP concentration in response to vasopressin [1]. The basal cell plasma membrane water permeability of AQP1 transfected cells was twofold greater than that of nontransfected cells, whereas the permeability of AQP2 transfected cells increased significantly only after vasopressin treatment [11].

No previous studies on the transepithelial water permeability in cells transfected with aquaporins had been reported. In previous experiments involving AQP2 [11], the cells were grown on glass coverslips so that transepithelial permeability could not be measured. In this

study, the net transepithelial water fluxes (Jw) were recorded every minute, employing a specially developed experimental device [2]. The results indicate that transfection with AQP2, but not with AQP1 (and to a lesser extent with a chimeric construction of AQP1 and AQP2 containing the C-terminus of AQP2) leads, in LLC-PK1 cells, to the development of a functional, vasopressin sensitive, transepithelial water pathway. AQP2 insertion occurs in such a way that it allows the reconstitution of the physiological hydrosmotic response in a polarized epithelial cell layer, comparable to the one observed in a native ADH-sensitive-water-channel-containing epithelial barrier (the toad urinary bladder).

## Materials and Methods

### Cell Culture, Plasmid Construction and Cell Transfection

LLC-PK1 cells were grown on Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco-BRL), in a 5% $CO_2$ atmosphere at 37°C. To construct the AQP1/2 chimera, rat AQP1 cDNA encoding the first 231 amino acids flanked by a Pstl site at the 3′ end was amplified by PCR from a rat AQP1 cDNA template. Nucleotides that encode 41 amino acids of the C-terminus of AQP2 were excised with Pstl and Xbal from rat AQP2 cDNA. The 123 base pair AQP2 fragment was ligated in frame with the 693 base pair AQP1 PCR product. The chimeric construct was subcloned in pCDNA3 vector (Invitrogen) and sequenced for expression in mammalian cells. Rat AQP1 and AQP2 expression vectors were constructed as described previously [11].

LLC-PK1 cells were plated at a density of $10^5$ cells in 35-mm dish and maintained in ME H-21 + 10% fetal bovine serum until they were 70% confluent. Cells were transfected (by Calcium phosphate-DNA precipitation) with 2 μg of cDNA and stably transfected cell colonies were obtained by growing cells in selection medium containing 500 μg/ml of Geneticin (G418 Gibco-BRL). Four types of stably transfected LLC-PK1 cell lines were employed in permeability measurements: (1) Control cells (mock, vector alone); (2) Cells expressing AQP1; (3) Cells expressing AQP2 (with c-myc epitope tag); and (4) Cells expressing the chimera of AQP1 with C-terminus of AQP2. The transfected cells were grown in DMEM medium containing G418 (100 μg/ml), and seeded ($2 \times 10^5$ cells) on Transwell filters (Costar, 24-mm diameter, 3-μm porous bottom). Confluence was observed at 10 days after seeding. Transepithelial resistance and potential difference were measured before mounting with a Millicell-ERS electric resistance system (Millipore). The electrical resistance of the system (90 ± 6 Ω) was routinely subtracted.

### Water Permeability Measurements

#### *In Transfected Cell Layers*

To perform permeability measurements in the transfected cells, the Transwell holders, their bottom covered with the confluent cell layer, were directly inserted between two Lucite chambers (*see* Fig. 1*A*) [17]. This defined apical (mucosal, 22 ml) and basal (serosal, 15.7 ml) compartments which were mixed continuously with magnetic stirring bars. Both sides of the monolayer were bathed with the same buffer solution (in mM): 116 NaCl, 25 $NaHCO_3$, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 2.4 $KH_2PO_4$, 0.4 $K_2HPO_4$, 1.8 KCl, 10 glucose; 37°C. The serosal bath was continuously bubbled with a 95% $O_2$/5% $CO_2$ mixture (pH 7.4 ± 0.1).

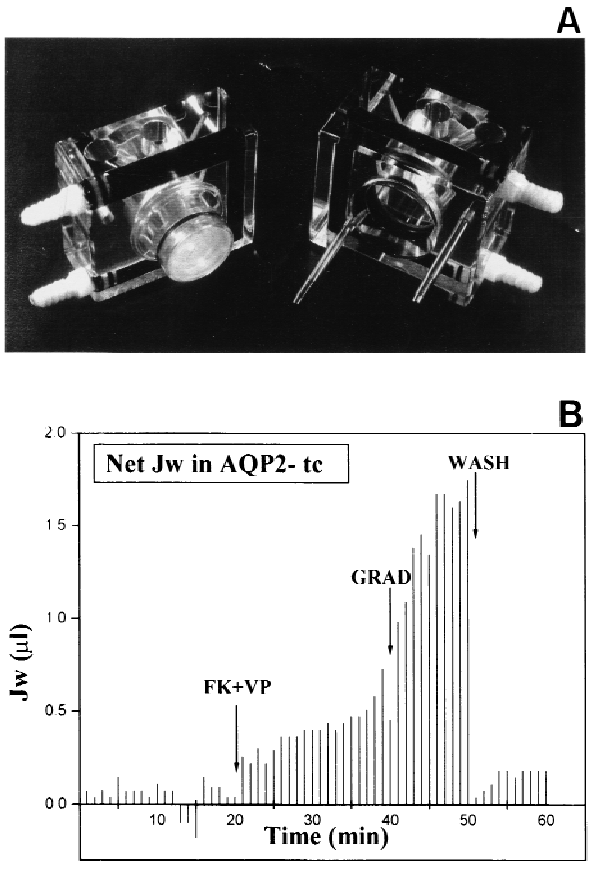


**Fig. 1.** (*A*) View of Transwell holder inserted in a modified Üssing chamber. (*B*) Screen captured from a typical experiment: Transepithelial net water flux (Jw) as a function of time in AQP2 transfected cells. FK + VP: forskolin (10 μM) and vasopressin (26 nM) were simultaneously added to the serosal bath. GRAD: 12.5 mM mannitol was added to the serosal bath; WASH: both the pharmacological agents and the gradient were simultaneously washed.

The net water flux (Jw) was recorded minute by minute as described elsewhere [2, 18]. Briefly, the position of a liquid meniscus inside a capillary tube was photoelectrically detected. Displacements to the right or to the left were proportional to the amount of water absorbed or secreted by the epithelial layer. The data were computed in units of $\mu l \cdot min^{-1}$. A hydrostatic pressure (ΔP) of 4.5 cm of water was applied continuously to the mucosal side. The sensitivity of the system was 50 nl [2], and the 3 μm pore size of the polycarbonate filter did not represent a significant barrier to water movement. Figure 1*B* shows a screen captured from a typical experiment. The height of each bar represents the amount of water moving across the tissue in one minute. Upperside values indicate absorption; lower side values indicate secretion.

#### *In Toad Urinary Bladders*

Urinary bladders were dissected from pithed toads (*Bufo arenarum*) and mounted as a diaphragm between two Lucite chambers. Both sides

**Table 1.** Transepithelial resistance measured before the experiment and initial Jw ($Jw_{in}$)

| Cellular type | Resistance | $Jw_{in}$ |
|---|---|---|
| | ($\Omega \cdot cm^2$) | ($\mu l \cdot min^{-1} \cdot cm^{-2}$) |
| Mock ($n = 5$) | 323 ± 13 | 0.02 ± 0.01 |
| AQP1 ($n = 5$) | 836 ± 72* | 0.20 ± 0.12 |
| AQP1/2 ($n = 7$) | 427 ± 56 | 0.08 ± 0.06 |
| AQP2 ($n = 7$) | 244 ± 20* | −0.06 ± 0.09 |

Mean ± SEM; AQP1, AQP1/2 (a chimera construction) and AQP2 transfected cells. *$P < 0.05$ when compared with Mock (control) values.

of the tissue were bathed with a solution containing (in mM): 112 NaCl, 1 $CaCl_2$, 2.5 $NaHCO_3$, 5 KCl, bubbled with air at 20°C. The transepithelial Jw was measured and recorded as described above. A ΔP of 10 cm of water was applied to the mucosal side.

Transepithelial osmotic gradients were obtained by adding mannitol to the serosal buffer. In the case of transfected cells, different osmotic gradients were tested. The small gradient of 12.5 mM used for studies here was selected to minimize cell volume changes, possible cell trauma, and to ensure full reversibility (50 mM were employed in toad urinary bladders). Forskolin (FK) and [$Arg^8$]-vasopressin (VP) were purchased from Sigma.

### Unidirectional $^{14}$C-mannitol Fluxes

In some experiments, $^{14}$C-mannitol was added to the apical side (0.5 μCi/ml) immediately before the start of the Jw measurements. Samples (1 ml) were taken from the basolateral side every 3 min. The mannitol permeability was calculated taking into account corrections for sampling dilution and back fluxes.

### Light and Electron Microscopy Studies

For microscopy, cells cultured on filters were fixed with 2% glutaraldehyde as previously described [18] and postfixed with a 1% $OsO_4$ solution. Samples were dehydrated in graded series of alcohol and embedded in Epone.

## Results

### Transepithelial Resistance and Basal Jw in Transfected Cell Layers

Table 1 shows the transepithelial resistance measured when the tested cells became confluent (10 days in culture) and immediately before mounting in the flux measurement chamber. When compared to mock transfected cells, AQP1 transfected cells showed higher resistance values. However, cells transfected with AQP2 showed significant lower values. The initially observed Jw ($Jw_{in}$) was, after stabilization (15 min), not significantly different from zero, indicating good epithelial tightness.

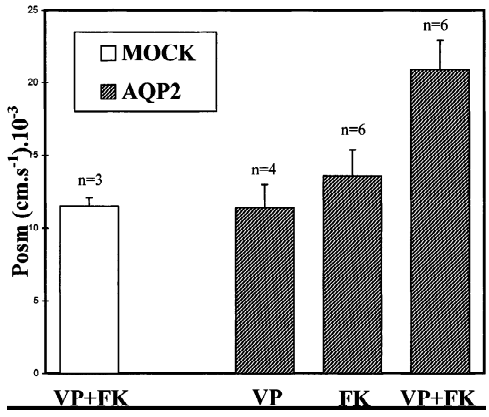


**Fig. 2.** Osmotic permeability of Mock and AQP2 transfected LLC-PK1 cell layers, treated with VP (26 nM), FK (10 μM) or VP + FK (osmotic gradient: 12.5 mM serosal mannitol). *Posm under VP + FK was significantly higher in AQP2 transfected cells when compared with the control ones (MOCK, $P < 0.01$) or when compared with AQP2 transfected cells treated with VP or FK alone ($P < 0.02$).

### Osmotic Permeability in Control Conditions and Under the Action of Forskolin and Vasopressin

Figure 2 shows the effect of VP, FK and both agents together on the osmotic permeability of AQP2 transfected cells. Results are compared with those observed in mock cells. Confluent epithelia were initially mounted between two isosmotic solutions and, after Jw stabilization, 26 nM vasopressin, 10 μM forskolin, or both agents together were added to the serosal bath. Next an osmotic gradient was created by increasing serosal osmolarity by 12.5 mM (mannitol). Finally, the osmotic gradient and the tested pharmacological agent(s) was (were) simultaneously washed. Mean of initial and final values in nonstimulated, isotonic conditions were taken as control values. It was observed that only when both drugs were simultaneously added was there a significant increase in Posm. This permeability increase was also significantly higher than that measured in the presence of FK or VP alone.

Based on these initial results (*see* Discussion), the next studies were carried out with both FK and VP added together in the four cell lines. Results were compared to control experiments where basal Posm (without FK and VP stimulation and after Jw stabilization) were measured. The results are summarized in Table 2. A clear increase in the transepithelial osmotic permeability under FK plus VP was observed in AQP2 transfected cells but not in the AQP1 transfected ones. In the case of the AQP1/2 cells (chimera) the FK + VP induced Posm increase was smaller than in AQP2 cells but significantly higher than in mock cells at rest.

To be sure that the paracellular pathway was not involved in the observed increases in Jw, unidirectional

**Table 2.** Jw and Posm (mean ± SEM) measured in Mock, AQP1, AQP1/2 and AQP2 transfected cells

| Cellular type | ΔJw with FK + VP (1) | Posm control (2) | Posm experimental (3) |
|---|---|---|---|
| | ($\mu l \cdot min^{-1}$) | ($cm \cdot sec^{-1} \cdot 10^{-3}$) | ($cm \cdot sec^{-1} \cdot 10^{-3}$) |
| Mock ($n = 5$) | 0.22 ± 0.13 | 6.7 ± 1.0 | 7.4 ± 4.8 |
| AQP1 ($n = 5$) | 0.26 ± 0.21 | 13.4 ± 3.6 | 15.3 ± 3.6 |
| AQP1/2 ($n = 6$) | 0.25 ± 0.11 | 10.8 ± 2.6 | 18.1 ± 4.8* |
| AQP2 ($n = 7$) | 1.00 ± 0.45 | 12.5 ± 3.3 | 39.9 ± 8.2** |

(1) Increase in Jw induced by FK (10 μM) plus VP (26 nM) in the absence of the osmotic gradient. (2) Posm measured in the presence of the gradient alone (12.5 mM mannitol in the serosal side). (3) Posm measured with the same gradient but after FK + VP stimulation. * $P < 0.05$ when compared with Mock control values. ** $P < 0.01$ when compared with AQP2 control values.

$^{14}$C-mannitol fluxes were measured, in parallel with the Jw measurements in some experiments. The results are summarized in Table 3 and Fig. 3. It is noted that the increases in Jw values did not parallel increases in mannitol permeability, either in the absence or in the presence of an osmotic gradient.

### Effects of Mercurial Agents on the Forskolin Plus Vasopressin-induced Response

It is well established that mercurial agents block the AQP2 water channel [5, 8, 27]. The effects of $HgCl_2$ on the AQP2 transfected cells are presented in Fig. 4. and Table 4. The experimental protocol was again similar to that employed in previous series. Experiments were performed in the absence or in the presence (both sides) of 0.3 mM $HgCl_2$. Interestingly, it was found that FK + VP induced an increase in Jw in the absence of an osmotic gradient (Pooling results presented in Table 2 and Table 3; the difference is also significant (0.83 ± 0.25 $\mu l \cdot min^{-1}$, $P < 0.001$). Concerning $HgCl_2$, the hydrosmotic response to FK plus VP was clearly inhibited. A significant reduction was also observed either in the basal Jw as in the nonosmotic FK + VP flux. In these experiments the measured Jw came back to basal values when the gradient and the pharmacological agents were removed (Fig. 4).

### Comparing the Response to Forskolin Plus Vasopressin in AQP2 Transfected Cell Layers and in the Toad Urinary Bladder

Figure 5 shows experiments in which the experimental protocol employed with AQP2 transfected epithelial layers (*shown in* Fig. 4) was applied to the toad urinary bladder, a recognized ADH-sensitive water channel containing epithelial barrier. The evolution of Jw was similar (*compare* Fig. 4 and 5): (1) FK + VP induced an increase in Jw in the absence of an osmotic gradient. (2) A secondary Jw increase accompanied the establishment of the osmotic gradient. (3) Full reversibility was observed after removal of the osmotic gradient and the pharmacological stimulus. (4) $HgCl_2$ inhibited, in both cases, the basal Jw, the nonosmotic response to FK + VP, and the hydrosmotic response to them.

### Electron Microscopy

Figure 6 (*A*1 and *A*2) shows the transfected cells forming a confluent monolayer on the permeable support. The cells do not invade the filter pores. Microvilli are present at the mucosal border, indicating a well-defined polarized phenotype. *A*2, *B*1 and *B*2 show AQP2 transfected cells, fixed in the absence or in the presence of an osmotic gradient (12.5 mM mannitol, serosal hypertonic). In the last situation, the development of spherical intracellular vacuoles is observed. Elongated structures are also seen in some cases. Figure 7 (*C*1 and *C*2) shows AQP2 transfected cells, stimulated with FK plus VP, and under an osmotic gradient. Vacuoles are quite frequent and virtually fill the cytoplasm. Nevertheless, tight junctions remained sealed and intercellular spaces are closed or only slightly open (*C*2). These results are compared (Fig. 7, *D*1 and *D*2) with those previously reported in the isolated epithelium of the frog urinary bladder (*see* Discussion).

## Discussion

This report presents the first measurements of transepithelial water permeability in aquaporin transfected cells. Cells grown on a permeable support provide a useful tool to study water movements, having a single cellular type and under a relatively simple topological arrangement

**Table 3.** $^{14}$C-mannitol permeability (Ps) and Jw values in AQP2 transfected cells (Mean ± SEM; N = 6)

| | Initial | FK + VP | Gradient | Wash |
|---|---|---|---|---|
| Jw ($\mu$l.min$^{-1}$) | −0.37 ± 0.06 | 0.61 ± 0.11** | 1.37 ± 0.09*** | 0.76 ± 0.32** |
| Ps (cm · sec$^{-1}$ · 10$^{-6}$) | 1.38 ± 0.40 | 2.33 ± 0.34 | 3.22 ± 0.82 | 3.96 ± 0.79* |

* $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$

Initial: Observed values in the absence of neither an osmotic gradient nor pharmacological stimulation. FK + VP: forskolin (10$\mu$M) and vasopressin (26 nM) were simultaneously added to the serosal bath. GRADIENT: Mannitol, 12.5 mM was added to the serosal bath; WASH: both the pharmacological agents and the gradient were simultaneously washed.

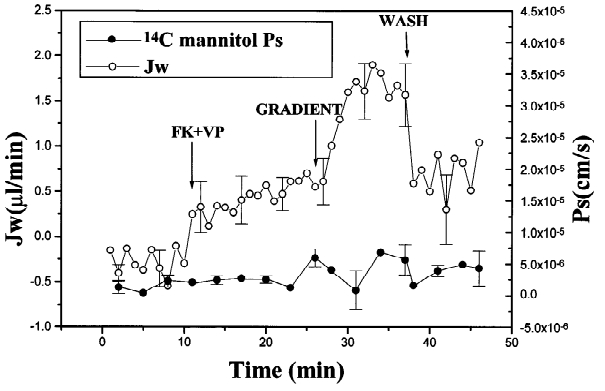


**Fig. 3.** Mean curves ($n = 6$) showing the simultaneous evolution of Jw and the mannitol permeability (Ps) in AQP2 transfected cell layers. FK + VP: forskolin (10 $\mu$M) and vasopressin (26 nM) were simultaneously added to the serosal bath. GRADIENT: 12.5 mM mannitol was added to the serosal bath; WASH: both the pharmacological agents and the gradient were simultaneously washed.

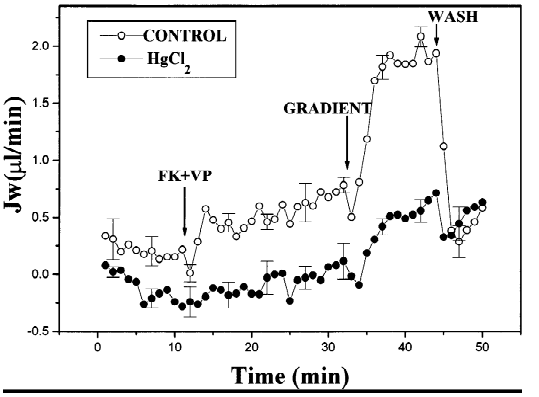


**Fig. 4.** Mean curves ($n = 6$) showing the evolution of Jw in AQP2 transfected cell layers in the absence (CONTROL) and in the presence of $HgCl_2$ (0.3 mM, both sides). Experimental conditions as in Fig. 3.

[17, 18]. They are then adequate to test the functional results of transfection experiments in epithelial cells.

AQP2 [5, 15] is selectively expressed in kidney and is localized in intracellular vesicles and in the apical membrane of collecting duct cells [4]. It has been reported as proposed previously [26], that vasopressin increases water permeability of kidney collecting ducts by inducing translocation of water channels (AQP2) to the plasma membrane [15]. Cytoplasmic microtubules play an important role in this process [20]. The packaging, sorting and selective delivery of proteins to the plasma membrane of all cells occur via two different pathways: the constitutive or nonregulated pathway and the regulated or stimulate one. To study these mechanisms, LLC-PK1 and HCD cells were stably transfected with the cDNA encoding AQP2 [11, 23]. The transfected channel is located mainly in a population of intracellular vesicles but it was recruited to the plasma membrane after vasopressin treatment or after an increase in intracellular cAMP by forskolin. This recruitment was accompanied by a significant increase in the mercurial-sensitive water permeability of these cells [11]. Vasopressin action induced a regulated transepithelial increase in water permeability. Present results demonstrate that vectorial increase in water permeability occurred in an epithelial layer formed by LLC-PK1 cells after transfection with AQP2 and, to a lesser extent, with an AQP1/AQP2 chimera.

## Reconstitution of a Regulated Water Pathway in Aquaporin-2 Transfected Epithelial Cells

Water movements across epithelial barriers can occur either across or between cells. The basal Jw was, in the four tested cell lines, not significantly different from zero. This indicated a good water epithelial tightness. Interestingly, the AQP1 transfected cells formed an epithelial barrier showing a transepithelial resistance higher than the control one. This result must be further investigated.

No differences in basal Posm among the three tested transfected lines (AQP1, AQP2, AQP1/2) were observed. Results presented in Table 2 and 4, and in Fig. 4 show that the combined effect of forskolin and vasopressin induced a significant increase in Jw in the AQP2

**Table 4.** AQP2 transfected cells

| Condition | Jw initial ($\mu l.min^{-1}$) | ΔJw with FK + VP ($\mu l.min^{-1}$) | ΔJw gradient ($\mu l.min^{-1}$) | Posm ($cm.sec^{-1}.10^{-3}$) | Posm corrected ($cm.sec^{-1}.10^{-3}$) |
|---|---|---|---|---|---|
| Control ($n = 6$) | 0.14 ± 0.14 | 0.38 ± 0.12* | 1.65 ± 0.20 | 27.6 ± 3.28 | 20.91 ± 2.02 |
| with $HgCl_2$ ($n = 5$) | −0.24 ± 0.08 | −0.10 ± 0.08 | 0.25 ± 0.11 | 4.1 ± 1.8 | |
| Control *vs.* $HgCl_2$ | $P < 0.05$ | $P < 0.01$ | $P < 0.001$ | $P < 0.001$ | |

* $P < 0.05$ *vs.* zero

Jw and Posm values in the absence (control) and in the presence of $HgCl_2$ (0.3 mM). Jw Initial: *Observed values in the absence of neither an osmotic gradient nor pharmacological stimulation*. ΔJw with FK + VP: Increase in Jw induced by forskolin (10 μM) and vasopressin (26 (nM) simultaneously added to the serosal bath. ΔJw gradient: Total increase in Jw observed when mannitol, 12.5 mM was added (maintaining FK + VP stimulation) to the serosal bath. Posm: the corresponding osmotic permeability values. Posm corrected: The nonosmotic increase in Jw generated by FK + VP was subtracted to calculate Posm.

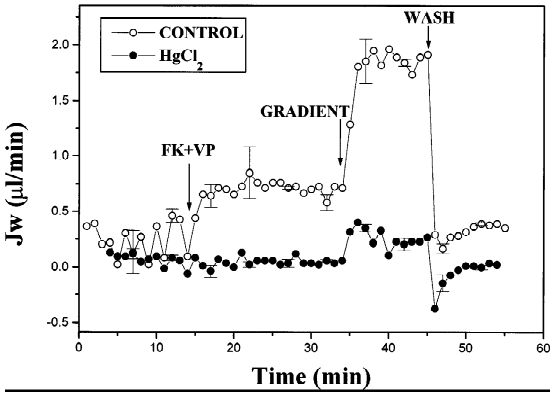


**Fig. 5.** Mean curves ($n = 3$) showing the evolution of Jw in toad urinary bladders in the absence (CONTROL) and in the presence of $HgCl_2$ (0.3 mM, both sides). Experimental conditions as in Fig. 3. (GRADIENT: 50 mM mannitol).

transfected cells, but not in AQP1 transfected ones. The increase was reversible, blocked by mercurial agents and did not parallel an increase in mannitol permeability.

The transfected epithelia were rather fragile. It was not possible to remove the apical or the basal baths more than once while maintaining full reversibility. In these conditions, it was difficult to evaluate the effects of $HgCl_2$ on cultured epithelia. If $HgCl_2$ is added only to one side of the epithelium, a transepithelial potential difference appeared, probably indicating $Hg^{++}$ adsorption to the membrane surface. This was associated with a nonreversible increase in water and mannitol permeability. The problem was circumvented by adding $HgCl_2$ to both sides of the epithelium. Furthermore, previous experiments demonstrated, both in transfected cells [11] as in amphibian urinary bladder [8] that $HgCl_2$ blocks the water channel.

The morphological studies demonstrated that the cells formed a single confluent layer. The appearance of intracellular vacuoles, under FK + VP and under an osmotic gradient is reminiscent of similar changes observed in AQP2 target tissues. In Fig. 7 *C* and *D*, the ultrastructural changes induced by FK + VP in AQP2 transfected cells can be compared with the morphological changes previously observed in the isolated epithelium of the frog urinary bladder, either at rest or under the action of ADH [19]. In both cases, plenty of vacuoles can be observed in the cytoplasm of the target cells, while the tight junction structure remains unmodified.

The FK + VP induced Posm increase in the case of the AQP1/2 cells (chimera) was smaller than in AQP2 cells but significantly higher than in mock cells at rest. These results suggest a role for the C-terminus in the AQP2 transfer to the apical membrane. Nevertheless, further experiments are necessary to confirm this working hypothesis.

VP alone (or FK alone) did not increase significantly Posm in AQP2 transfected cells. This is at variance with the results observed in cells cultured on a nonpermeable support [11]. The discrepancy with the results presented here could be due to several possible reasons. In Katsura et al. experiments [11] as well as in Valenti et al. experiments [23] vasopressin action was tested in the presence of a rather high osmotic gradient (100 mOsm). We employed here a much more reduced one (12.5 mOsm). Furthermore, we were measuring transepithelial fluxes and not, as in previous studies, the time course of cell volume changes after an osmotic challenge on the mucosal side. In polarized epithelial cells a direct relationship between the time course of cell volume changes and transepithelial permeability is not necessarily observed [16]. Katsura et al. also reported that AQP2 channels were both transferred to the apical and the basolateral membranes [11]. Nevertheless if, in our experimental conditions, functional AQP2-channels are mainly transferred to the apical membrane a limited increase in transepithelial water permeability would not be unexpected. In physiological conditions resident water channels are present in the basolateral membrane of vasopressin-target cells. Two aquaporins, AQP3 [3] and AQP4 [6, 9, 22] have been reported to be located in the basolateral membrane of collecting ducts. It must be remarked that

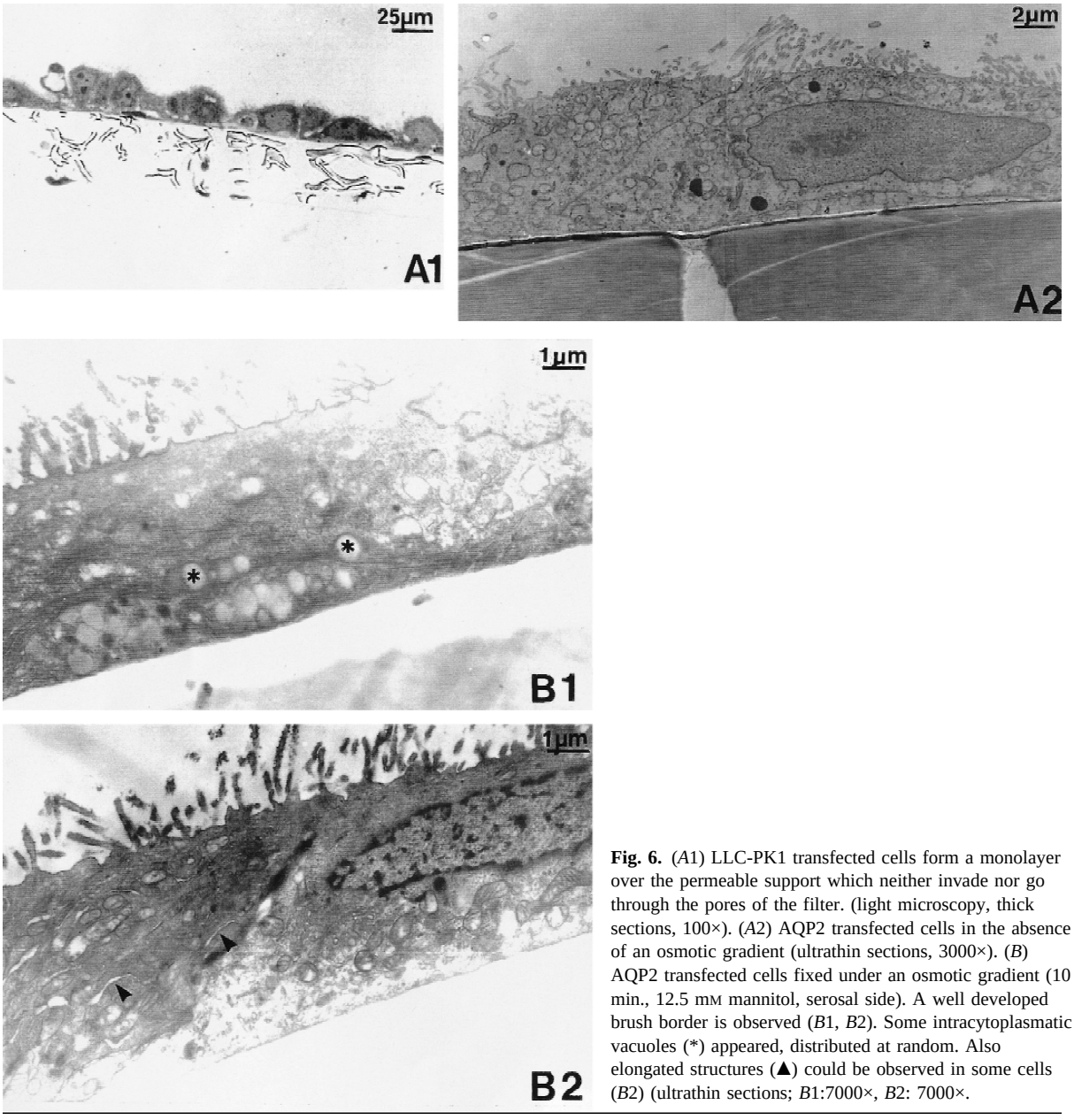


**Fig. 6.** (*A*1) LLC-PK1 transfected cells form a monolayer over the permeable support which neither invade nor go through the pores of the filter. (light microscopy, thick sections, 100×). (*A*2) AQP2 transfected cells in the absence of an osmotic gradient (ultrathin sections, 3000×). (*B*) AQP2 transfected cells fixed under an osmotic gradient (10 min., 12.5 mM mannitol, serosal side). A well developed brush border is observed (*B*1, *B*2). Some intracytoplasmatic vacuoles (*) appeared, distributed at random. Also elongated structures (▲) could be observed in some cells (*B*2) (ultrathin sections; *B*1:7000×, *B*2: 7000×.

we tested the reversibility of the hydrosmotic response in all experiments. Furthermore, the response to FK + VP in AQP2 cells was compared against two different control conditions: (1) stimulated Mock cells and (2) non-stimulated AQP2 transfected cells.

Direct demonstration of AQP2 water channel recycling in the stable transfected LLC-PK1 epithelial cells was recently reported [10]. We have now used the same cellular model. The similarity observed between the evolution of Jw in the toad urinary bladder, a well known ADH-sensitive water channel containing epithelial barrier, and in the AQP2 transfected epithelial cells was striking. Several points must be mentioned: (1) The combined action of FK plus VP induced a similar hydrosmotic response in the toad urinary bladder and in the AQP2 transfected epithelium. (2) $HgCl_2$ inhibited, in both cases, the hydrosmotic response to FK + VP. (3) A significant increase in Jw induced by FK + VP, in the absence of a transepithelial osmotic gradient, also appeared in AQP2 transfected epithelia and in the toad urinary bladder. It was not paralleled by an increase in the $^{14}$C-mannitol permeability and it was also inhibited

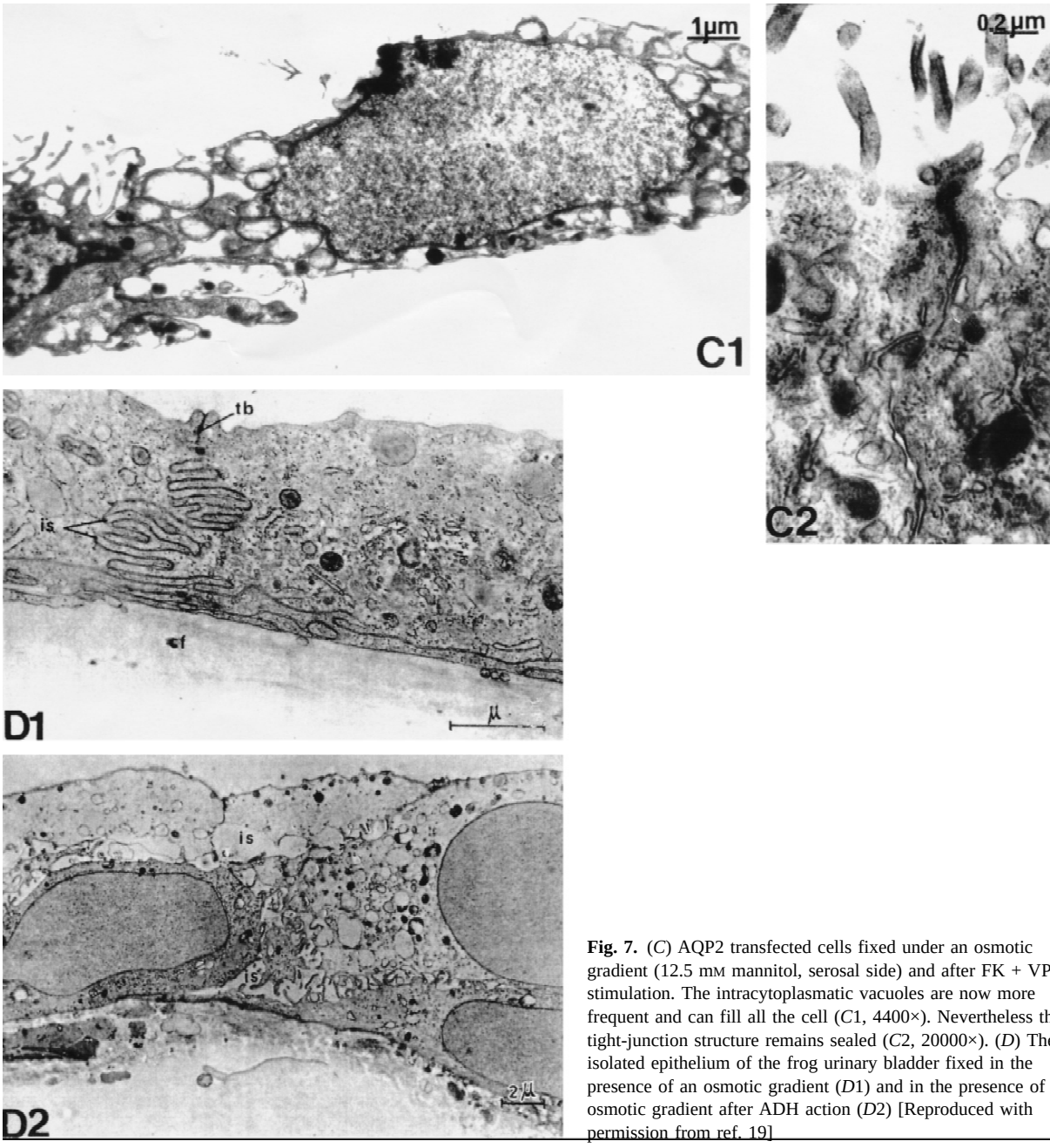


**Fig. 7.** (*C*) AQP2 transfected cells fixed under an osmotic gradient (12.5 mM mannitol, serosal side) and after FK + VP stimulation. The intracytoplasmatic vacuoles are now more frequent and can fill all the cell (*C*1, 4400×). Nevertheless the tight-junction structure remains sealed (*C*2, 20000×). (*D*) The isolated epithelium of the frog urinary bladder fixed in the presence of an osmotic gradient (*D*1) and in the presence of an osmotic gradient after ADH action (*D*2) [Reproduced with permission from ref. 19]

by $HgCl_2$. These results can be interpreted as a transcellular water movement associated with a solute transport. Nevertheless, despite the findings with $^{14}C$ mannitol, a role for a paracellular flow driven by the small mucosal hydrostatic gradient, can not be completely excluded. (4) The similarity between the responses to FK plus VP in the AQP2 transfected cells and in the toad urinary bladder as well as the similar effect of $HgCl_2$ in both cases, allow us to consider that the functional reconstitution of the vasopressin-regulated transepithelial water pathway was achieved.

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