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# Characterization of the ion transport responses to ADH in the MDCK-C7 cell line

**Abstract** The Madin-Darby canine kidney (MDCK) cell line expresses many characteristics of the renal collecting duct. The MDCK-C7 subclone forms a high-resistance, hormone-responsive model of the principal cells, which are found in distal sections of the renal tubule. The electrophysiological technique of short-circuit current measurement was used to examine the response to antidiuretic hormone (ADH) in the MDCK-C7 clone. Three discrete electrogenic ion transport phenomena can be distinguished temporally and by the use of inhibitors and effectors. Initially the cells exhibit anion secretion through the cystic fibrosis transmembrane conductance regulator (CFTR). The presence of CFTR was confirmed by immunoprecipitation followed by Western blotting. The CFTR-mediated anion secretion is transient and is followed, in time, by a verapamil- and $Ba^{2+}$-sensitive anion secretion or cation absorption and, finally, by $Na^+$ reabsorption via epithelial $Na^+$ channels (ENaC). In contrast to other studies of MDCK cells, we see no indication that the presence of CFTR functionally inhibits ENaC. The characterization of the various ion transport phenomena substantiates this cell line as a model renal epithelium that can be used to study the hormonal and metabolic regulation of ion transport.

**Key words** Amiloride · Cystic fibrosis transmembrane regulator · Electrogenic transport · Epithelial sodium channel · Potassium channels · Principal cells · Renal epithelia · Verapamil

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## Introduction

The Madin-Darby canine kidney (MDCK) cell line has been widely used as a model for the study of vectorial protein sorting in polarized epithelial cells and, more recently, as a transfection model for protein expression. This cell line has many of the characteristics of the epithelial cells lining the distal portion of the renal nephron [22, 32] but the parent line shows cellular heterogeneity and several laboratories have produced subclones with various characteristics [9, 15, 16, 19, 21, 22, 32, 34]. The laboratory of Dr. H. Oberleithner has isolated two subclones. The C7 line has many of the characteristics of the principal cells while the C11 subclone demonstrates characteristics of intercalated cells [9].

The MDCK-C7 cells form a monolayer in culture which develops a high-resistance ($>1000 \, \Omega \, cm^2$) phenotype. We have characterized the natriferic responses of this epithelium to aldosterone, insulin-like growth factor 1 (IGF1) and antidiuretic hormone (ADH) in cells grown on permeable supports, and found that confluent monolayers have hormone-mediated $Na^+$ transport responses that are similar to those described for the mammalian distal nephron [2].

In subsequent studies using the C7 subclone to dissect the signal transduction pathways involved in hormonal stimulation, it became apparent that these cells have multiple electrogenic transport processes. This is consistent with the expectation of transport phenomena in the mammalian distal tubule and distinguishes this cell line from the amphibian models of principal cells, such as the A6 cell line and the toad urinary bladder. The complex nature of the transport processes in MDCK-C7 cells makes them a more applicable model in which to study reported interactions between transporters in response to a single stimulus.

Since there is a lack of well-characterized distal tubule cell lines, many previous studies have utilized transfection techniques, where the transport proteins of interest have been overexpressed and/or expressed in heterologous cells. In either case, the normal regulatory compo-

nents of the transport process may be ineffective. To characterize a model system which endogenously expresses several transporters, we have examined, in detail, the cellular response to ADH in the MDCK-C7 cell line. We determined that there are least three distinct electrogenic transport processes which can be temporally separated.

## Materials and methods

### Materials

The hormones and inhibitors used in these experiments were: ADH ([arg]-vasopressin, Sigma, St. Louis, Mo., USA); amiloride hydrochloride (Sigma); DASU-01 (synthesized and donated by Dr. R. Bridges, University of Pittsburgh, Pa., USA); 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB; Biomol Research, Plymouth Meeting, Pa., USA); barium chloride (BaCl$_2$; Sigma); and verapamil hydrochloride (ICN Biomedical, Aurora, OH, USA). The antibodies used for immunoprecipitation and Western blotting were monoclonal mouse anti-human cystic fibrosis transmembrane conductance regulator (CFTR), R-domain-specific (Genzyme, Cambridge, Mass., USA) and peroxidase-conjugated goat IgG fraction to mouse IgG (Cappel Research, Durham, N.C., USA). Other supplies used in the immunoprecipitation were GammaBind Plus Sepharose (LKB Biotechnology, Uppsala, Sweden) and chemiluminescent detection reagents (Chemilum, New England Nuclear, Life Sciences, Boston, Mass., USA).

### MDCK-C7 cell culture

The MDCK-C7 cells were grown in a 37°C humidified incubator with a 5% CO$_2$ and 95% O$_2$ gas mixture. Initially the cells were grown in 75 cm$^2$ flasks and fed with Minimal Media with Earle's salts, non-essential amino acids and L-glutamine (MEM; Gibco/BRL, Grand Island, New York, USA) supplemented with 10% fetal bovine serum (Sigma), 26 mM NaHCO$_3$ and adjusted to pH 7.0. The confluent cells were subcultured by trypsinization and the cells were seeded (5.4×10$^4$ cell/cm$^2$) onto Nucleopore polycarbonate membranes forming the bottom of Transwell chambers (Costar, Cambridge, Mass., USA). The Transwell chambers were placed in specially designed tissue culture plates to form a two-compartment system in which media are added to both apical and basolateral surfaces. The media were aspirated and replaced three times per week. The cells were used between passages 73 and 96.

### Electrophysiological studies

Nucleopore filters (4.7 cm$^2$) containing confluent MDCK-C7 cells (7–12 days) were removed from the Transwell chambers and clamped between the halves of an Ussing chamber (World Precision Instruments, Sarasota, Fla., USA). Each half of the chamber contained a tapered fluid compartment with openings for voltage electrodes (close to the epithelial membrane) and current electrodes (at the opposite end of the chamber). The fluid chamber was water jacketed to maintain constant temperature (37°C). The cells were bathed in serum-free MEM. The media were circulated in the chambers by means of a 5%CO$_2$/O$_2$ gas lift. The electrodes were connected to a voltage-clamp amplifier (Current Voltage Clamp; World Precision Instruments) for measurement of net ion flux as monitored under short-circuit conditions (SCC; short circuit current) [31]. Transepithelial resistance was calculated by applying a 2-mV pulse across the epithelium and measuring the resultant deflection in SCC. Data from cultures were used only if they maintained a resistance >1000 Ω cm$^2$.

The cultures were placed in the Ussing chambers and incubated under short-circuited conditions until a steady baseline transport was achieved (0.5–1 h). The cultures were preincubated with inhibitors, where appropriate, for 30 min prior to the addition of ADH. ADH was added to the serosal bathing media; amiloride was added to the apical media 30 min after ADH to determine the portion of the transport due to flux through the amiloride-sensitive Na$^+$ channel. The concentrations of effectors used were: ADH, 0.1 IU/ml; amiloride, 30 μM; NPPB, 500 μM; DASU-01, 500 μM; BaCl$_2$, 3 mM; and verapamil, 25 μM. Each experiment was performed using matched cultures grown in parallel. The data are presented as means ±SE with $n$ indicating the number of different experiments. Comparisons were performed using Student's one-tailed $t$-test for paired samples; values of $P<0.05$ were considered significant.

### CFTR immunoprecipitation and Western blotting

To prepare total cell extracts for identification of CFTR, the MDCK-C7 cells were scraped from the Transwell supports using serum-free medium, pelleted and washed once by centrifugation at 1200 $g$. The cell pellet was homogenized in homogenizing
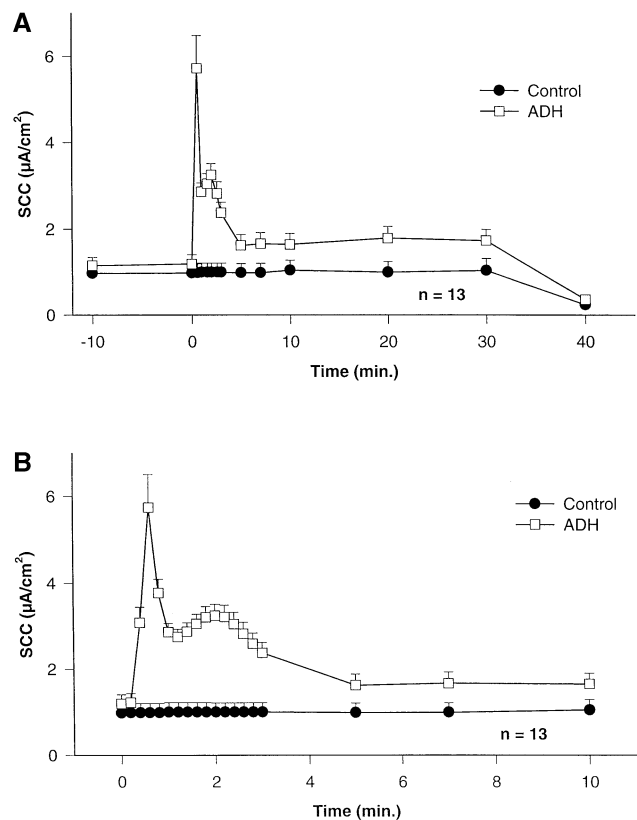


**Fig. 1A,B** Ion transport response of MDCK-C7 cells to stimulation by [arg]-vasopressin (*ADH*). Ion transport is measured as short-circuit current (*SCC*). A positive deflection indicates cation movement from the apical to serosal bathing medium or anion movement from serosal to apical medium. Where indicated, ADH (100 mU/ml) was added to the serosal bathing medium at time zero. After 30 min, amiloride (10$^{-5}$ M) was added to the apical solution. **A** The entire 30-min time course of the experiment as well as 10 min of the stabilization period (–10–0 min) and the 10 min after the addition of amiloride (30–40 min). **B** The same data but only showing the first 10 min of the ADH response with additional time points to better illustrate the multiphasic nature of the response. *Symbols* denote the mean ±SEM. *n*=number of paired experiments. Between 4 and 30 min the means of the ADH-treated samples were significantly different from control samples ($P<0.05$)
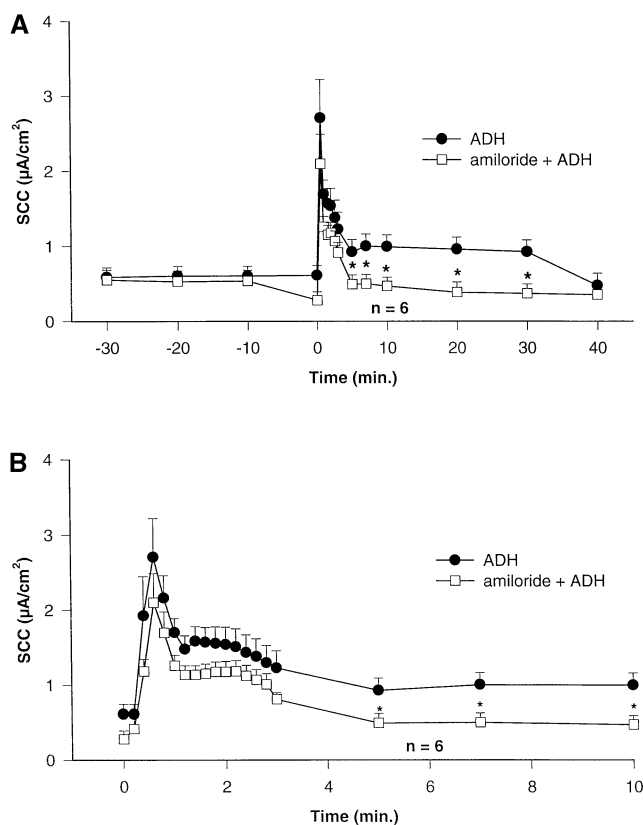
**A**

**B**

**Fig. 2A,B** Ion transport response of MDCK-C7 cells to stimulation by ADH in control and amiloride-pretreated cells. Ion transport is measured as short circuit current (*SCC*). A positive deflection indicates cation movement from the apical to serosal bathing medium or anion movement from serosal to apical medium. Where indicated, amiloride was added to the apical medium at –10 min. After a 10-min preincubation, ADH (100 mU/ml) was added to the serosal bathing medium at time zero. After 30 min, amiloride ($10^{-5}$ M) was added to the apical solutions. **A** The entire time course of the experiment. **B** The same data but only showing the first 10 min of the ADH response with additional time points. *Symbols* denote the mean ±SEM. *n*=number of paired experiments. The means of the amiloride-treated samples were significantly different from control samples (\**P*<0.05) at the time points indicated in **A**

buffer (50 mM TRIS/1% Triton X-100, pH 7.5) using 40 strokes of a Dounce tight-fitting homogenizer. The homogenate was spun in a microfuge (16,000 *g*) and the supernatant containing the solubilized proteins was incubated with mouse anti-CFTR antibody (1:1000 dilution) for 1 h at room temperature. GammaBind Plus Sepharose beads were added to bind the antigen/antibody complexes and the incubation was continued on a rotary stirrer for 2 h at room temperature. The beads were pelleted (16,000 *g*) and washed five times with wash buffer (0.3% NP-40, 0.3% sodium deoxycholate, 0.3% NaCl, 50 mM TRIS, pH 7.4) with 5-min incubations on the rotary stirrer between washes. The wash buffer washes were followed by three washes with deionized water. After the final wash, the pelleted beads were extracted by a 30-min incubation with sodium dodecyl sulfate (SDS) lysis buffer (50 mM TRIS, pH 6.8, 2% SDS, 120 mM dithiothreitol, 10% glycerol). The 16,000 *g* supernatant from the extraction was separated by one-dimensional polyacrylamide gel electrophoresis (1D-PAGE) using a 7.5% separatory gel and 4% stacking gel. After electrophoresis, the separated proteins were electrophoretically transferred to PVDF (polyvinylidene fluoride) blotting membranes.
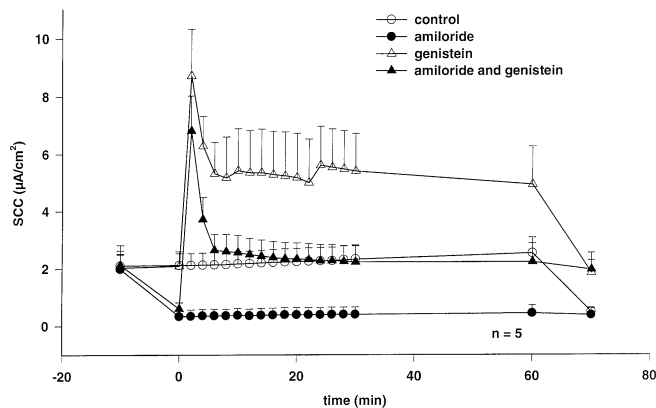


**Fig. 3** Ion transport response of MDCK-C7 cells to stimulation by genistein in control and amiloride-pretreated cells. Ion transport is measured as short-circuit current (*SCC*). A positive deflection indicates cation movement from the apical to serosal bathing medium or anion movement from serosal to apical medium. Where indicated, amiloride was added to the apical medium at –10 min. After a 10-min preincubation, genistein (40 µg/ml) was added bilaterally at time zero. After 60 min, amiloride ($10^{-5}$ M) was added to all apical solutions. *Symbols* denote the mean ±SEM. *n*=number of paired experiments

The PVDF-immobilized protein blots were blocked with 1% bovine serum albumin to prevent non-specific binding and probed using a CFTR antibody (monoclonal mouse anti-human CFTR R-domain specific) followed by binding of a secondary horseradish peroxidase IgG (goat anti-mouse). The specific binding was detected using a chemiluminescent detection method (Chemilum) according to the manufacturer's instructions.

## Results

ADH has been previously shown to stimulate an increase in amiloride-sensitive $Na^+$flux in the MDCK-C7 subclone measured both 60 and 120 min after the addition of the hormone [2]. However, stimulation by the peptide hormone (100 mU/ml) also leads to an immediate ion transport response, with a rapid transient peak that occurs in the first minute. This is followed by an apparent second peak that reaches a transport maximum approximately 6 min after the addition of hormone and then declines. Finally, when compared to control, there is a sustained, low level of increased transport (Figure 1). In a limited number of experiments, stimulation by 1 mU/ml ADH produced similar results (data not shown).

Our previous data [2] suggested that the sustained action of ADH was due entirely to $Na^+$ absorption through the amiloride-sensitive epithelial $Na^+$ channel, ENaC. To determine whether the more rapid transport events are also mediated via the $Na^+$ transport pathway, cells were pretreated with amiloride for 10 min before the addition of ADH (Fig. 2). Interestingly, amiloride appeared to block only the sustained transport seen at the later time points and had no effect on the first two transport peaks. Thus, it appears that ADH stimulates several transport events in the MDCK-C7 cells.
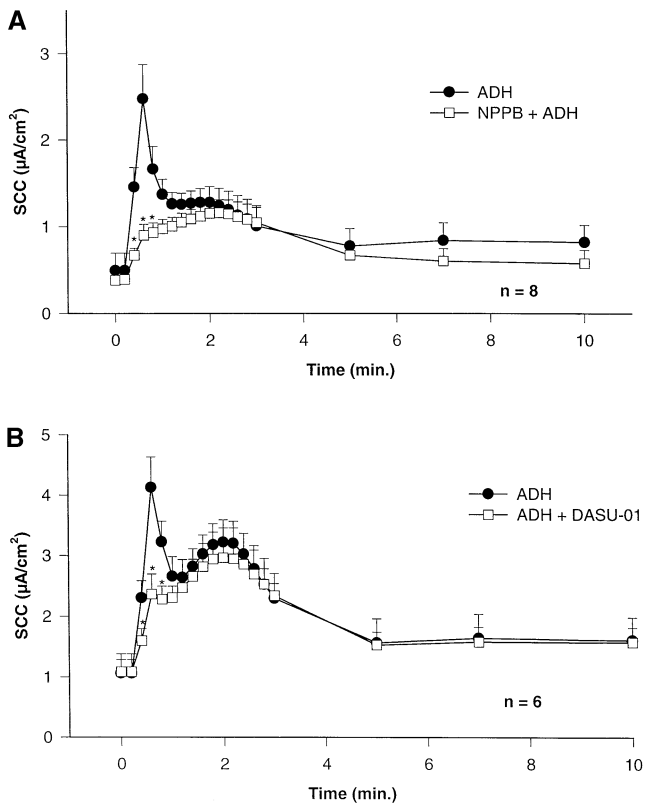
**Fig. 4A,B** Ion transport response of MDCK-C7 cells to stimulation by ADH in cells pretreated with inhibitors of cystic fibrosis transmembrane conductance regulator (*CFTR*) ion flux. Ion transport is measured as short-circuit current (*SCC*). A positive deflection indicates cation movement from the apical to serosal bathing medium or anion movement from serosal to apical medium. Inhibitors (NPPB, 500 µM added apically; DASU-01, 500 µM, added bilaterally) were added 30 min before the addition of ADH (100 mU/ml) to the serosal bathing medium at time zero. In each case, only the first 10 min of the responses to ADH is shown. There was no significant difference between control and inhibitor-pretreated cells after 2 min. *Symbols* denote the mean ±SEM. *n*=number of paired experiments. The means of the inhibitor-pretreated samples were significantly different from control samples (*$P<0.05$) at the time points indicated

A possible mechanism for the initial increase in transcellular transport was suggested by treatment of the cells with genistein, an isoflavone which inhibits tyrosine kinases [27]. This compound has been shown to inhibit both insulin- and aldosterone-stimulated Na⁺ transport in the A6 cell line [20]. However, genistein has also been shown to stimulate an anion transport response via the CFTR protein ([23] and references contained therein). Treatment of the MDCK-C7 cells with genistein caused an immediate increase in transport, similar to the type of response elicited by ADH (Fig. 3). The genistein-stimulated transport was not due to an increase in Na⁺ because the magnitude of the response was maintained in cells which had been pretreated with amiloride.

These findings suggested that the initial transport response in the MDCK-C7 cells may be due to anion flux via CFTR. To explore this possibility, we tested the ef-
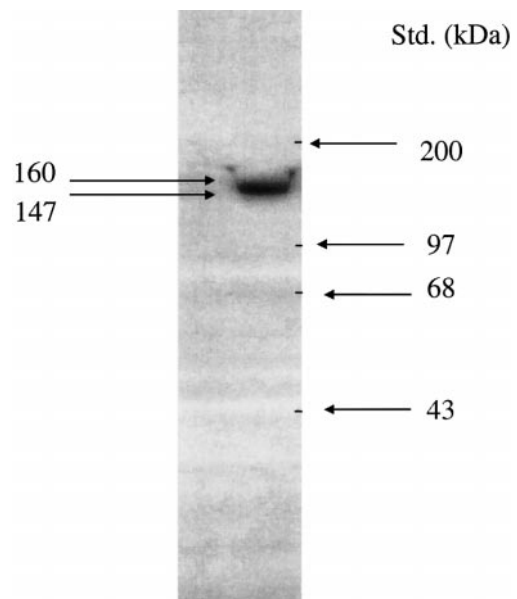


**Fig. 5** Western blot of CFTR immunopurified from MDCK-C7 cells. A whole-cell homogenate from MDCK-C7 cells was immunoprecipitated using an anti-CFTR antibody. The immunopurified sample was separated using polyacrylamide gel electrophoresis (7.5% separatory gel; 4% stacking gel), blotted onto PVDF membrane, probed with anti-CFTR antibody and detected by chemiluminescence. The single band between 147 and 160 kDa represents CFTR. The molecular weights of the standards run on the same gel are indicated to the *right* of the figure

fects of two inhibitors of CFTR, NPPB and the diarylsulfonylurea DASU-01, on the response to ADH. NPPB inhibits several transport pathways while diarylsulfonylureas are thought to be more specific inhibitors of CFTR [23]. Both of these compounds inhibited the rapid but transient ion transport response that is stimulated within the first minute after treatment with ADH, but did not appear to affect the second transport event (which has a maximal effect at approximately 2 min) (Fig. 4). These data suggest that the initial, rapid response to ADH is anion secretion via CFTR.

To confirm that the C7 subclone expresses the CFTR protein, a whole-cell homogenate was immunoprecipitated with a monoclonal antibody to CFTR and the antibody-bound fraction was separated by 1D-PAGE, transferred to PVDF blotting membrane and probed with the anti-CFTR antibody (Fig. 5). A single band of 147–160 kDa was visible after the Western blotting. This protein is consistent with the molecular weight of the CFTR protein and confirms the presence of this transporter in the MDCK cells.

Finally we sought to determine the nature of the ion flux responsible for the amiloride-NPPB- and DASU-01-insensitive component of the response to ADH. Verapamil, an inhibitor of L-type Ca²⁺ channels as well as a modulator of the inositol-1,4,5-trisphosphate receptor, IP₃R [18], inhibits this response suggesting a role for increased intracellular Ca²⁺ (Fig. 6A). Interestingly, this second component of the transport response is also sensi-
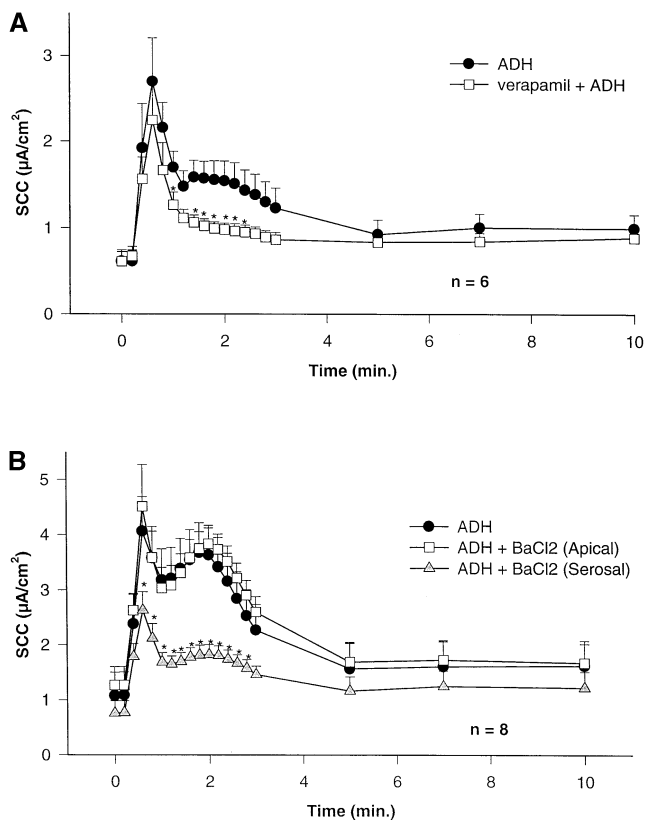
**Fig. 6** Ion transport response of MDCK-C7 cells to stimulation by ADH in cells pretreated with verapamil or Ba²⁺. Ion transport is measured as short-circuit current (*SCC*). A positive deflection indicates cation movement from the apical to serosal bathing medium or anion movement from serosal to apical medium. Inhibitors (verapamil, 25 μM, added bilaterally; Ba²⁺, 3 mM added as indicated) were added 30 min before the addition of ADH (100 mU/ml) to the serosal bathing solution at time zero. In each case, only the first 10 min of the responses to ADH is shown. There was no significant difference between control and inhibitor-pretreated cells after 4 min. *Symbols* denote the mean ±SEM. *n*=number of paired experiments. The means of the inhibitor-pretreated samples were significantly different from control samples (*$P<0.05$) at the time points indicated

tive to the addition of Ba²⁺ to the serosal but not apical bathing medium, suggesting a role for a K⁺ channel on the basolateral membrane (Fig. 6B). These results are consistent with the presence of a Ca²⁺-activated K⁺ channel.

## Discussion

Much of our basic understanding of hormonal control of salt and water transport comes from work done in amphibian model systems such as the toad urinary bladder, frog skin and the A6 cell line derived from the kidney of *Xenopus laevis*. This is, in part, because of a lack of mammalian cell culture models for the hormone-responsive principal cells found in the mammalian distal nephron and cortical collecting duct. One common problem in the development of such lines is a failure to develop a

"tight" or high-resistance phenotype that is found in the epithelial cells lining this section of the tubule. This makes measurement of transepithelial ion flux in intact monolayers difficult if not impossible. Another common problem that has been noted in cell lines that have been derived from SV40 large T-antigen immortalized renal cells is uncontrolled cell proliferation under permissive temperatures [29]. However, the MDCK-C7 cells used in these studies are relatively unique because the cell line exhibits contact inhibition after forming an electrically tight (1000–4000 Ω cm²) monolayer and, therefore, forms an ideal model for studying ion fluxes.

Previous work has shown that the MDCK-C7 line expresses ENaC and responds to the natriferic hormones, aldosterone, ADH and IGF1 [2]. In the current study, we have analyzed, in greater detail, the response to ADH and have characterized multiple transport responses (Fig. 1) that can be separated by time and use of inhibitors.

Greater than 50% of the basal current appears to be due to a constitutive level of Na⁺ reabsorption. When amiloride is added to cells, there is an immediate drop in SSC (Fig. 2, –10 to 0 time points). After the addition of ADH, there is an increase in Na⁺ transport but this appears to be a relatively late event which is manifested after two other transport events and is the only electrogenic ion flux that appears to be sustained for more than a few minutes (Fig. 2; [2]).

The nature of the initial transport response was suggested by the effect of genistein on the MDCK-C7 cells. Genistein, a tyrosine kinase inhibitor, causes an increase in Cl⁻ flux in several epithelial lines by activating CFTR [8, 12]. In our studies, genistein stimulated an increase in ion transport (Fig. 3) which could be inhibited by NPPB (data not shown), suggesting that genistein was also stimulating flux through CFTR in the MDCK-C7 cells.

To substantiate the possibility that the first transport event stimulated in response to ADH was anion secretion through CFTR, MDCK-C7 cells were pretreated with NPPB prior to stimulation with ADH. The NPPB completely blocked the initial transport response. At concentrations used in this experiment (500 μM), NPPB is not specific for CFTR and has been shown to effectively inhibit transport through other Cl⁻ channels such as the outwardly rectifying Cl⁻ channel (ORCC), Ca⁺-dependent Cl⁻ channel in airway epithelia and a volume-sensitive ORCC [23].

However, a more specific inhibitor of CFTR, the diarylsulfonylurea DASU-01, had exactly the same inhibitory effect on the initial transport response (Fig. 4B), suggesting that the ion flux represents anion secretion through CFTR.

In order to determine if the MDCK-C7 cells express CFTR, an immunoprecipitation was done using an anti-CFTR antibody. Immunoprecipitation followed by immunodetection showed a single band, consistent with the molecular weight of CFTR.

CFTR has been found in most sections of the nephron as well as in cells derived from patients with autosomal

dominant polycystic kidney disease [10]. For the purposes of this study, CFTR has been immunolocalized to the apical membrane of distal nephron segments [4]. CFTR has also been demonstrated in model renal cell lines of the inner medullary collecting duct of the mouse (mIMCD-K2) [33] and CFTR mRNA has been found in the M-1 line derived from mouse cortical collecting duct as well as in cells from freshly isolated rabbit cortical collecting duct [30]. Finally, CFTR is constitutively expressed in the apical membrane of the MDCK I cells, which have characteristics of distal nephron cells [16]. Therefore, our demonstration of CFTR in the MDCK-C7 cells is consistent with what is known about the expression of this protein in the kidney as well as other principal cell models.

The anion that is secreted in response to activation of CFTR is likely to be either $Cl^-$ or $HCO_3^-$. Previous work has demonstrated that either species can be transported by the CFTR channel in airway [7] as well as intestinal [11, 25] epithelia. The nature of the anion flux will be determined by the driving forces, predominately determined by transporters in the basolateral membrane [5, 7]. The MDCK-C7 cell line will provide a model system for future exploration of the transporters and driving forces that are likely to control the nature of the anion flux through CFTR in renal epithelia.

Anion flux through CFTR equilibrates rapidly and this initial transport is followed by a second response that is insensitive to both the CFTR inhibitors and amiloride. This second response is, however, inhibited by both $Ba^{2+}$ added to the serosal side or verapamil applied bilaterally (Fig. 6). $Ba^{2+}$ is a relatively non-selective inhibitor of $K^+$ channels while verapamil is known to be an inhibitor of L-type $Ca^{2+}$ channels as well as a down-regulator of the $IP_3R$ in the endoplasmic reticulum (ER). $IP_3$ binding to the ER receptor causes the release of $Ca^+$ through the $IP_3$ receptor-gated $Ca^+$ channel [18]. While L-type $Ca^{2+}$ channels have not, to our knowledge, been demonstrated in renal principal cells, the presence of $IP_3R$ in the distal nephron has been recently reported [18].

These results suggest the presence of a $Ca^{2+}$-dependent $K^+$ channel but do not define the nature of the increase in $Ca^{2+}$. The principal cells have been shown to express two receptors for ADH, the V2 receptor which causes an increase in cAMP by activating adenylate cyclase [13] and a V1 receptor that causes an increase in $IP_3$ [3].

Anion secretion via CFTR and $Na^+$ reabsorption via ENaC are stimulated by ADH binding to the V2 receptor on the basolateral side of the cell, triggering the production of cAMP by adenylate cyclase [13]. However, in the principal cells of the collecting duct, ADH can also bind the V1 receptor causing the production of $IP_3$ [3]. The resulting $Ca^+$ release can be down-regulated by prolonged stimulation by ADH or verapamil [26].

There are multiple $K^+$ channels which could contribute to the absorption of the important cation. Although we have been unable to characterize this pathway fully, several possibilities exist including channels which are
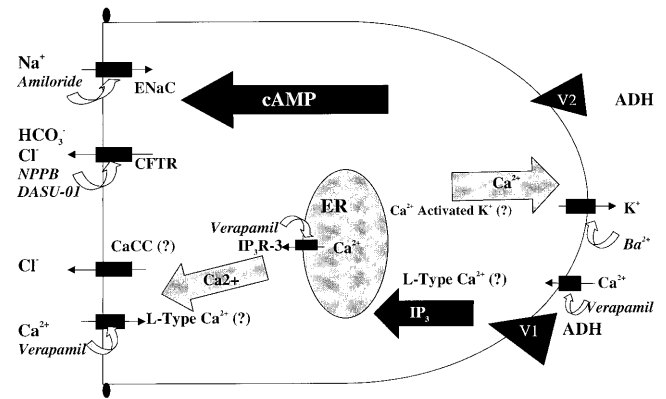


**Fig. 7** Hypothetical model of transporters in the MDCK-C7 cell line. This hypothetical model illustrates transporters that may be present in the MDCK-C7 cells to account for the data presented in this manuscript. The *left-hand side* of the figure represents the apical membrane, the *right* represents the basolateral membrane. *Rectangular shapes* indicate apical membrane or endoplasmic reticulum transporters. *Elliptical shapes* indicate basolateral membrane transporters. *Triangular* indicate receptors in the plasma membrane. *Curved arrows* indicate inhibitors of particular transporters shown at their proposed site(s) of action

directly activated by cAMP or indirectly activated by changes in intracellular $Ca^{2+}$ [5, 7]. Regulation of these channels forms an important branch point for the control of intracellular driving forces and can, for example, modulate flux through apically located channels such as CFTR [7], as well as contribute to the increased $K^+$ reclamation that is necessary during high $Na^+$ reabsorption.

To summarize the results, we have found the following transport responses to ADH in chronological order: anion secretion via CFTR, verapamil-sensitive, $Ba^{2+}$-sensitive anion secretion or cation absorption and, finally, $Na^+$ absorption via ENaC. From these results we propose the potential model of the MDCK-C7 cells in Fig. 7. The figure shows two ADH receptors, V1 and V2, and the two different pathways which could, theoretically, be involved. The cAMP pathway is known to activate both CFTR and ENaC while the $IP_3$ pathway activates $IP_3R$-3 causing the release of $Ca^{2+}$ from the ER. In this model we have included the L-type $Ca^{2+}$ channel and the $Ca^{2+}$-activated $Cl^-$ channel although there is no direct evidence that they are present.

Probably the most exciting aspect of these studies is that the characterization of the ADH response in the C7 cells can give a unique view of the endogenous interactions between CFTR and ENaC in a native epithelial cell. The properties of CFTR as a $Cl^-$ channel are well documented [6] but more recent studies have indicated that this protein may directly regulate other channels such as the outwardly rectifying $Cl^-$ channel [14] and ENaC [24, 28]. In one of the key experiments demonstrating that CFTR inhibits ENaC, a high-resistance subclone of the MDCK cell line was used to co-express the channels. MDCK cells were stably transfected with ENaC and then transiently transfected with CFTR. The ENaC-transfected cells respond to ADH stimulation with

an increase in Na$^+$ reabsorption while the cells transfected with both ENaC and CFTR showed no increase in amiloride-sensitive transport in response to the same stimulation [28]. The CFTR-mediated total inhibition of ENaC activity was not transient and was maintained for the length of the experiment. The authors concluded that the presence of CFTR functionally inhibited the ability of ENaC to transport Na$^+$ in response to ADH [28].

Transporting epithelial cells endogenously express very low numbers of functional ENaC and CFTR channels – typically 50–100 channels per cell [1, 16, 17]. The electrophysiological data in our current studies show that the number of functional channels found in the MDCK-C7 cells falls within this range (calculations not shown – see [1] for conversion chart). The total number of CFTR channels that are present in cells could, however, be variable. Whether the transporters measured in these studies are inserted into the membrane in response to ADH or are pre-existing and activated by a phosphorylation event is unknown. Regardless of the mechanism of activation, there is no indication that the presence of CFTR functionally inhibits ENaC. Rather the two ion transporters are both stimulated in response to ADH, albeit with different time courses. This discrepancy between a lack of functional inhibition when the CFTR and ENaC are expressed at endogenous levels and the profound and complete inhibition of ENaC by CFTR when both are overexpressed in transfected MDCK cells remains unexplained. The major difference between the two situations is, of course, the number of expressed channels. Previous studies have shown that hyperexpression of CFTR alters its regulation and physiological properties [17]. Resolution of this enigma will require additional experimentation.

The characterization of the ADH response in the non-transfected MDCK-C7 clonal line will provide a better understanding of the complexity of hormone effects on the principal cells of the distal nephron. These cells will also be a useful model with which to examine the transport and metabolic effects that govern the nature of the anion which is secreted by CFTR and the interaction of this channel with other transporters.

# References

1. Blazer-Yost BL, Helman SI (1997) The amiloride-sensitive epithelial Na$^+$ channel: binding sites and channel densities. Am J Physiol 272:C761–C769
2. Blazer-Yost BL, Record RD, Oberleithner H (1996) Characterization of hormone-stimulated Na$^+$ transport in a high-resistance clone of the MDCK cell line Pflügers Arch 432:685–691
3. Burnatowska-Hledin MA, Spielman WS (1989) Vasopressin V1 receptors on the principal cells of the rabbit cortical collecting tubule. J Clin Invest 83:84–89
4. Crawford I, Maloney PC, Zeitlin PL, Guggino WB, Hyde SC, Turley H, Gatter KC, Harris A, Higgins CF (1991) Immunocytochemical localization of the cystic fibrosis gene product CFTR. Proc Natl Acad Sci USA 88:9262–9266
5. Cuthbert AW, Hickman ME, Thorn P, MacVinish LJ (1999) Activation of Ca$^{2+}$ and cAMP-sensitive K$^+$ channels in murine colonic epithelia by 1-ethyl-2-benzimidazolone. Am J Physiol 277:C111–C120
6. Dawson DC, Smith SS, Mansoura MK (1999) CFTR: mechanism of ion conduction. Physiol Rev Suppl 79:S47–S75
7. Devor DC, Singh AK, Lambert LC, DeLuca A, Frizzell RA, Bridges RJ (1999) Bicarbonate and chloride secretion in Calu-3 human airway epithelial cells. J Gen Physiol 113:743–760
8. French PJ, Bijman J, Bot AG, Boomaars WEM, Scholte BJ, DeJonge HR (1997) Genistein activates CFTR Cl$^{-+}$ channels via a tyrosine kinase- and proteins phosphatase-independent mechanism. Am J Physiol 273:C747–C753
9. Gekle M, Wunsch S, Oberleithner H, Silbernagl S (1994) Characterization of two MDCK-cell subtypes as a model system to study principle and intercalated cell properties. Pflügers Arch 428:157–162
10. Hanaoka K, Devuyst O, Schwiebert EM, Wilson PD, Guggino WB (1996) A role for CFTR in human autosomal dominant polycystic kidney disease. Am J Physiol 270:C389–C399
11. Illek B, Fischer H, Machen TE (1998) Genetic disorders of membrane transport II. Regulation of CFTR by small molecules including HCO$_3^-$. Am J Physiol 275:G1221–G1226
12. Illek B, Fischer H, Santos GF, Widdicombe JH, Machen TE, Reenstra WW (1995) cAMP-independent activation of CFTR Cl channels by the tyrosine kinase inhibitor genistein. Am J Physiol 268:C886–C893
13. Jard S (1998) Vasopressin receptors: a historical survey. Adv Expert Med Biol 449:1–13
14. Jovov B, Ismailov I, Benos DJ (1995) Cystic fibrosis transmembrane conductance regulator is required for protein kinase A activation of an outwardly rectified anion channel purified from bovine tracheal epithelia. J Biol Chem 270:1521–1528
15. Kersting U, Schwab A, Treidtel M, Pfaller W, Gstraunthaler G, Steigner W, Oberleithner H (1993) Differentiation of Madin-Darby canine kidney cells depends on cell culture conditions. Cell Physiol Biochem 3:42–55
16. Mohamed, A, Ferguson D, Seibert FS, Cai H-M, Karnter N, Grinstein S, Riordan JR, Lukacs GL (1997) Functional expression and apical localization of the cystic fibrosis transmembrane conductance regulator in MDCK I cells. Biochem J 322:259–265
17. Mohammad-Panah R, Demolomb S, Riochet D, Leblais V, Loussouarn G, Pollard H, Baro I, Escande D (1998) Hyperexpression of recombinant CFTR in heterologous cells alters its physiological properties. Am J Physiol 274:C310–C318
18. Monkawa T, Hayashi M, Miyawaki A, Sugiyama T, Yamamoto-Hino M, Hasegawa M, Furuichi T, Mikoshiba K, Saruta T (1997) Localization of inositol 1,4,5-triphosphate receptors in the rat kidney. Kidney Int 53:296–301
19. Nakazato Y, Suzuki H, Saruta T (1989) Characterization of subclones of Madin-Darby canine kidney renal epithelial cell line. Biochim Biophys Acta 1014:57–65
20. Record RD, Johnson M, Lee S, Blazer-Yost BL (1996) Aldosterone and insulin stimulate amiloride-sensitive sodium transport in A6 cell by additive mechanisms. Am J Physiol 271:C1079–C1084
21. Richardson JCW, Scalera V, Simmons NL (1981) Identification of two strains of MDCK cells which resemble separate nephron tubule segments. Biochim Biophys Acta 673:26–36
22. Sair MH Jr (1981) Growth and differentiated properties of a kidney epithelial cell line (MDCK). Am J Physiol 240:C106–C109
23. Schutz BD, Singh AK, Devor DC, Bridges RJ (1999) Pharmacology of CFTR chloride channel activity. Physiol Rev Suppl 79:S109–S144
24. Schwiebert EM, Benos DJ, Egan ME, Stutts MJ, Guggino WB (1999) CFTR is a conductance regulator as well as a chloride channel. Physiol Rev Suppl 79:S145–S166

25. Seidler U, Blumenstein I, Kretz A, Viellard-Baron D, Rossman H, Colledge WH, Evans M, Ratcliff R, Gregor M (1997) A functional CFTR protein is required for mouse intestinal cAMP-, cGMP- and $Ca^{2+}$-dependent $HCO_3^-$ secretion. J Physiol (Lond) 505:411–423

26. Sipma H, Deelman L, DeSmedt H, Missiaen L, Parys JB, Vanlingen S, Henning RH, Casteels R (1998) Agonist-induced down-regulation of type 1 and type 3 inositol 1,4,5-tris-phosphate receptors in A7r5 and $DDT_1$ MF-2 smooth muscle cells. Cell Calcium 23:11–21

27. Srinivas PR, Grunberger G (1994) Inhibitors of the insulin receptor tyrosine kinase. Pharmacol Ther 64:23–35

28. Stutts MJ, Canessa CM, Olsen JC, Hamrick M, Cohn, JA, Rossier BC, Boucher RC (1995) CFTR as a cAMP-dependent regulator of sodium channels. Science 269:847–849

29. Takacs-Jarrett M, Sweeney WE, Avner ED, Cotton CU (1998) Morphological and functional characterization of a conditionally immortalized collecting tubule cell line. Am J Physiol 275: F802–F811

30. Todd-Turla KM, Rusval E, Naray-Fejes-Toth A, Fejes-Toth G (1996) CFTR expression in cortical collecting duct cells. Am J Physiol 270:F237–F244

31. Ussing HH, Zerahn K (1951) Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta Physiol Scand 23:110–127

32. Valentich JD (1981) Morphological similarities between the dog kidney cell line MDCK and the mammalian cortical collecting tubule. Ann NY Acad Sci 372:384–405

33. Vandorpe D, Kizer N, Ciampollilo F, Moyer B, Karlson K, Guggino WB, Stanton BA (1995) CFTR mediates electrogenic chloride secretion in mouse inner medullary collecting duct (mIMCD-K2) cells. Am J Physiol 269:C683–C689

34. Wunsch S, Gekle M, Kersting U, Schuricht B, Oberleithner H (1995) Phenotypically and karyotypically distinct Madin-Darby canine kidney cell clones respond differently to alkaline stress. J Cell Physiol 164:164–171