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Role of CFTR's PDZ1-binding domain, NBF1 and Cl^- conductance in inhibition of epithelial Na^+ channels in *Xenopus* oocytes

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Abstract

The cystic fibrosis transmembrane conductance regulator (CFTR) inhibits epithelial Na^+ channels (ENaC). Evidence has accumulated that both Cl^- transport through CFTR Cl^- channels and the first nucleotide binding domain (NBF1) of CFTR are crucial for inhibition of ENaC. A PDZ binding domain (PDZ-BD) at the C-terminal end links CFTR to scaffolding and cytoskeletal proteins, which have been suggested to play an important role in activation of CFTR and eventually inhibition of ENaC. We eliminated the PDZ-BD of CFTR and coexpressed Na^+/H^+-exchange regulator factors together with CFTR and ENaC. The results do not support a role of PDZ-BD in inhibition of ENaC by CFTR. However, inhibition of ENaC was closely linked to Cl^- currents generated by CFTR and was observed in the presence of Cl^-, I^- or Br^- but not gluconate. Therefore, functional NBF1 and Cl^- transport are required for inhibition of ENaC in *Xenopus* oocytes, while the PDZ-BD is not essential.

Keywords: Cystic fibrosis transmembrane conductance regulator; Epithelial Na^+ channel; *Xenopus* oocyte; Mouse trachea; Cystic fibrosis; PDZ1; Na^+/H^+-exchange regulator factor; Tyrosine kinase activating protein; cAMP; Epithelial transport; Nucleotide binding fold

1. Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP regulated Cl^- channel and a regulator of other channels [1]. The best examined and pathophysiologically probably most relevant example is the inhibition of amiloride sensitive epithelial Na^+ channels (ENaC) by CFTR [1]. Due to the CFTR defect and the lack of inhibition of ENaC, amiloride sensitive Na^+ transport is enhanced in both airways and colon of CF patients [2–4]. However, in other epithelial tissues such as the sweat duct, the situation is remarkably different. Here, CFTR has been demonstrated to act as an activator rather than an inhibitor of ENaC channels [5]. The reasons for these obviously tissue specific effects of CFTR on ENaC remain currently unknown. Recent

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; IBMX, 3-isobutyl-1-methylxanthine; ENaC, epithelial Na^+ channel; NHERF1, Na^+/H^+-exchange regulator factor; TKA-1, tyrosine kinase activating protein

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results suggest participation of additional proteins in the CFTR/ENaC interaction. These unknown proteins might be expressed differentially in colon, airways and sweat duct, thereby generating tissue specific regulatory relationships.

It has been shown previously that CFTR contains a common site for protein interaction at the very C-terminal end of the protein, a so called PDZ binding domain (PDZ-BD). Binding of regulatory proteins to this C-terminal end of CFTR like the Na^+/H^+-exchange regulator factor (EBP50, NHERF1, NHERF2) has been demonstrated [6]. This PDZ domain has been shown to be essential for proper expression of CFTR in the apical plasma membrane [7,8]. CFTR is anchored in the apical membrane via cytoskeletal interaction using the ezrin binding phosphoprotein EBP50 [9]. Moreover, ezrin may function as an A kinase anchoring protein thereby linking protein kinase A to CFTR which is essential for protein kinase A (PKA) mediated phosphorylation and activation of CFTR [10]. The NHERF1 homologue E3KARP (NHERF2, TKA-1, SIP-1) mediates the association of ezrin and PKA with CFTR in airway cells [11]. Both NHERF1 and NHERF2 have been shown recently to bind to β_2-adrenergic receptors as well as P2Y receptors and may therefore be multifunctional adopter proteins essential for intracellular signaling [12]. These results suggest that NHERF proteins might also participate in the regulation of ENaC by CFTR. We therefore examined whether the presence of an intact C-terminal CFTR PDZ-BD is essential for activation of CFTR and the interaction with ENaC in *Xenopus* oocytes.

Apart from the inhibition of ENaC, CFTR has been demonstrated to interact with a diversity of other membrane conductances [13,14]. For some of these interactions, the requirement of an intact first nucleotide binding domain of CFTR has been demonstrated [15–17]. In the present paper we show that inhibition of ENaC largely depends on the CFTR Cl^- current, which is activated during stimulation of CFTR. Using a number of CFTR first nucleotide binding domain (NBF1) mutants, we show that downregulation of ENaC does not take place in the absence of CFTR Cl^- channel activity. Both properties of CFTR, namely that of a Cl^- channel and a regulator of ENaC, are therefore intimately related.

2. Materials and methods

2.1. cRNAs of NHERF1, NHERF2, ENaC and CFTR

cDNAs encoding mouse NHERF1 and human NHERF2 (TKA-1) were kindly obtained from Prof. Dr. E. Weinman, University of Maryland, USA and Prof. Dr. A. Ullrich, Max Planck Institut, Martinsried, Germany. The three (α, β, γ) subunits of the rat amiloride inhibitable Na^+ channel ENaC were kindly provided by Prof. Dr. B. Rossier, Pharmacological institute of Lausanne, Switzerland. The triple mutant NHERF1-S288/290/291A and the truncation NHERF1-E130X were generated by standard polymerase chain reaction (PCR) techniques. Using similar PCR techniques, the NBF1 mutants of human CFTR ΔF508, G551D, S466L, K464A, D572N, KH483/484AA, R487Q, R516A, KR598/600GA, KK611/612AA and K615A were in vitro synthesized (Quickchange, Stratagene). Proper sequences were confirmed by automatic sequencing (Big Dye Sequencing Kit). cDNAs encoding mutant and wild type (wt) NHERF1, NHERF2, CFTR and ENaC were linearized at the 3′ end using *Kpn*I, *Hin*dIII, *Xho*I, *Not*I and *Hpa*I and were in vitro transcribed using SP6, T3 and T7 polymerases and a 5′cap (mMessage mMachine, Ambion, USA).

2.2. Expression in Xenopus oocytes

Isolation and microinjection of oocytes have been described in a previous report [15]. In brief, after isolation from adult *Xenopus laevis* female frogs (*Xenopus* Express, Cape Town, South Africa) oocytes were dispersed and defolliculated by a 0.5 h treatment with collagenase (type A, Boehringer, Germany). Subsequently, oocytes were rinsed and kept in ND96 buffer (in mmol/l): NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, Na-pyruvate 2.5, pH 7.55, supplemented with theophylline (0.5 mmol/l) and gentamicin (5 mg/l) at 18°C. Oocytes were injected with cRNA (1–10 ng) after dissolving cRNA in about 50 nl double distilled water (PV830 pneumatic pico pump, WPI, Germany). Oocytes injected with 50 nl double distilled water served as controls. Two to four days after injection oocytes were impaled with two electrodes (Clark instruments) which had

a resistance of 1 MΩ when filled with 2.7 mol/l KCl. A flowing (2.7 mol/l) KCl electrode served as bath reference in order to minimize junction potentials and had a resistance of 700 Ω. Membrane currents were measured by voltage clamping of the oocytes (OOC-1 amplifier, WPI, Germany) in intervals between −80 and +40 mV in steps of 20 mV for 1 s. Current data were filtered at 400 Hz (OOC-1 amplifier). Between intervals, oocytes were voltage clamped to −10 mV for 5 s. Data were collected continuously on a computer hard disk at a sample frequency of 1000 Hz and were analyzed using the programs chart and scope (McLab, AD-Instruments, Macintosh). During the experiments the bath was continuously perfused at a rate of 5–10 ml/min. All experiments were conducted at room temperature (22°C). Whole cell conductances generated by CFTR (G_{CFTR}) upon stimulation of the oocytes with 3-isobutyl-1-methylxanthine (IBMX; 1 mmol/l) and forskolin (2 μmol/l) were determined in the presence of amiloride (10 μmol/l). Amiloride sensitive conductance (G_{Amil}) was determined by subtracting the whole cell conductance determined in the presence of amiloride from the total conductance.

2.3. Materials and statistical analysis

All used compounds were of highest available grade of purity. IBMX, forskolin, and amiloride were all from Sigma (Deisenhofen, Germany). Ionomycin was from Calbiochem (Australia). NaBr, NaI, and gluconate were obtained from Merck (Darmstadt, Germany). Paired or unpaired Student's *t*-test and analysis of variance were used for statistical analysis. *P* values < 0.05 were accepted to indicate statistical significance (*).

3. Results

3.1. The C-terminal PDZ-BD of CFTR is not required for activation of CFTR or inhibition of ENaC in Xenopus oocytes

The PDZ-BD at the C-terminal end of CFTR was mutated in vitro (L1480V-CFTR) and coexpressed together with the epithelial Na^+ channel in *Xenopus* oocytes. Amiloride sensitive whole cell currents were assessed before and after stimulation of CFTR by IBMX (1 mM) and forskolin (2 μM). As demonstrated in Figs. 1 and 2, Na^+ currents inhibited by amiloride were largely reduced after stimulation of CFTR. Stimulation of oocytes expressing only ENaC did not change amiloride sensitive ENaC conductance (data not shown). A Cl^- conductance (G_{CFTR}) of similar magnitude was activated in both wtCFTR and L1480V-CFTR expressing oocytes upon stimulation with IBMX and forskolin (I/F). The inhibition of ENaC (G_{Amil}) during activation of CFTR was comparable for wt and mutant CFTR (Fig. 2B). A truncated version of CFTR was expressed, comprising the first transmembrane spanning domain, the first nucleotide binding domain and the R domain (E831X-CFTR). When stimulated with I/F, truncated CFTR generated a whole cell conductance which was significantly reduced when compared to wtCFTR. Nevertheless, a small but significant portion of G_{Amil} was inhibited during stimulation of E831X-CFTR (Fig. 2). In addition, we generated a CFTR mutant which only lacks the last six amino acids, encoding the PDZ-BD (E1474X-CFTR). Much to our surprise,

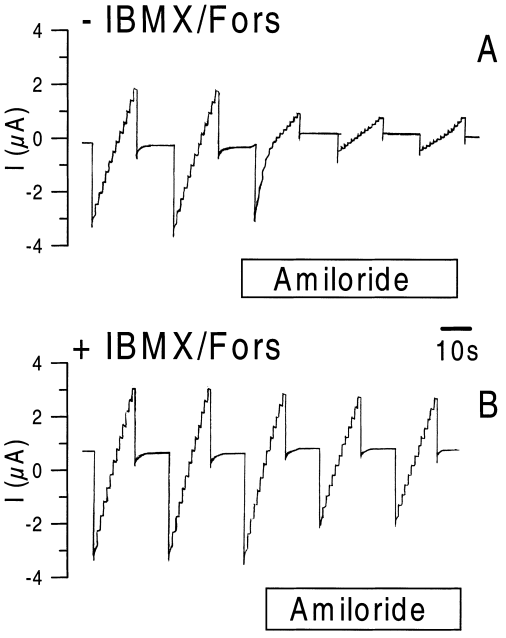

Fig. 1. Original recordings of the whole cell currents measured in a *Xenopus* oocyte coexpressing L1480V-CFTR and ENaC. Effect of amiloride before (A) and after (B) stimulation with IBMX (1 mM) and forskolin (2 μM). Oocytes were voltage clamped in intervals from −90 to +30 mV.

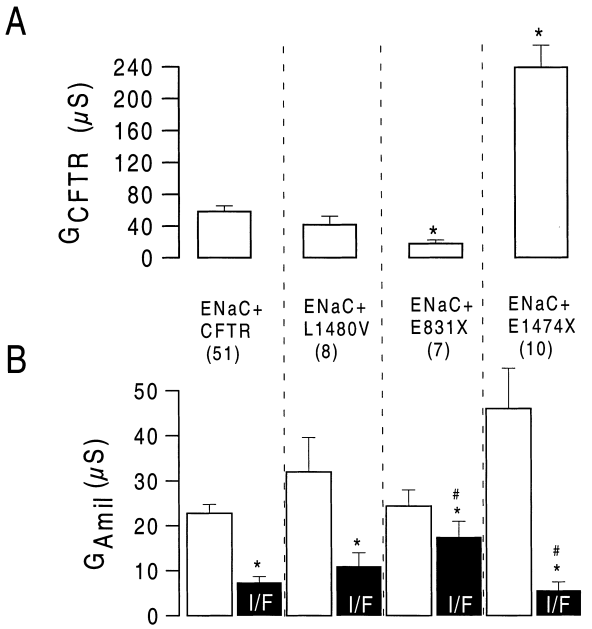

Fig. 2. Coexpression of wtCFTR, L1480V-CFTR, E831X-CFTR and E1474X with ENaC. Summary of CFTR Cl^- conductances (G_{CFTR}) *Significant changes in activation of G_{CFTR} when compared with wtCFTR (A) and amiloride sensitive whole cell conductances (G_{Amil}) (B) before and after stimulation with IBMX and forskolin (I/F). *Significant inhibition of amiloride sensitive conductance. Stimulation of either wtCFTR, L1480V-CFTR, E831X-CFTR or E1474X significantly enhanced CFTR whole cell Cl^- conductance. Figures in parentheses indicate numbers of experiments. *Significant inhibition of G_{Amil}; #significant changes for CFTR dependent inhibition of G_{Amil} when compared with wtCFTR.

E1474X-CFTR generated a significantly larger current than wtCFTR and largely downregulated G_{Amil} (Fig. 2). In fact, downregulation of ENaC was significantly enhanced when compared to wtCFTR, which is likely due to the large Cl^- conductance generated by E1474X-CFTR. These results clearly indicate that the C-terminal PDZ-BD of CFTR is required neither for activation of CFTR nor for CFTR mediated inhibition of ENaC in *Xenopus* oocytes.

We further ruled out a role of PDZ-BD in the inhibition of ENaC, by coexpressing NHERF1 and NHERF2 with ENaC and CFTR. NHERF1 and NHERF2 have been demonstrated to interact with the CFTR PDZ-BD [6,11]. Coexpression of NHERF1 ($n=8$) or NHERF2 ($n=12$) with only ENaC had no impact on the magnitude of the Na^+ conductance expressed in the oocytes and did not confer any cAMP sensitivity to ENaC (data not shown). G_{CFTR} was activated by IBMX and forskolin (Fig. 3, gray bars), in the presence of NHERF1 or NHERF2. When compared with CFTR Cl^- conductance in the absence of NHERF (G_{H2O}), the magnitude was similar in the presence of the various NHERF constructs (Fig. 3). G_{Amil} was detected under all experimental conditions and was 35.6 ± 3.5 μS (G_{H2O}), 71.3 ± 6.3 μS (G_{NHERF1}), 33.3 ± 3.1 μS ($G_{NHERF1-E130X}$), 37.5 ± 3.7 μS ($G_{NHERF1-S288/290/291A}$), and 30.7 ± 2.9 μS (G_{NHERF2}) (Fig. 3). Moreover, CFTR was still able to downregulate ENaC in the presence of NHERF1 or NHERF2 and the downregulation was similar in the absence or presence of NHERF (% inhibition, black bars in Fig. 3). We further examined whether elimination of the putative phosphorylation domains in NHERF1 (NHERF1-S288/290/291A) or deletion of the second PDZ (PDZ2) domain of NHERF1 (NHERF1-E130X) impairs regulation of ENaC by CFTR. However, both mutants had no impact on activation of CFTR or inhibition of ENaC by CFTR. Thus, the PDZ domain proteins NHERF1 and NHERF2, which presumably interact with the CFTR PDZ-BD [11], have no impact on activation of CFTR or inhibition of ENaC by CFTR in *Xenopus* oocytes.

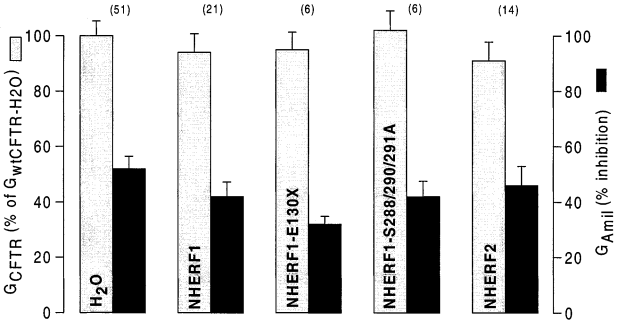

Fig. 3. Activation of CFTR Cl^- conductance (G_{CFTR}) and inhibition of amiloride sensitive ENaC conductance (G_{Amil}) in the absence or presence of NHERF1, NHERF2 and mutant NHERF1 (NHERF1-E130X, NHERF1-S288/290/291A). CFTR conductances (G_{CFTR}, gray bars) are expressed as fractions of the CFTR conductance activated in the absence of NHERF ($G_{CFTR-H2O}$). Inhibition of amiloride sensitive conductances (black bars) is expressed as % inhibition of the initial ENaC conductance ($G_{Amil-con} - G_{Amil-IBMX/Fors}/G_{Amil-con}$). Activation of CFTR whole cell Cl^- conductance and inhibition of ENaC by stimulation of CFTR was significant for all combinations. Figures in parentheses indicate numbers of experiments.

3.2. NBF1, Cl^- conductance and inhibition of ENaC

In contrast to the CFTR PDZ-BD, which does not seem to be essential for inhibition of ENaC by CFTR, at least in *Xenopus* oocytes, we have reported previously the requirement of an intact NBF1 of CFTR [15]. On the other hand, we and others have shown that Cl^- ions have to be present for inhibition of ENaC by CFTR [18,19]. In the present experiments, we aimed to identify the amino acid sequence within NBF1 that may play an essential role for inhibition of ENaC. To that end, we tried to generate CFTR-NBF1 mutants, which have lost their ability to activate a Cl^- conductance, but still inhibit ENaC or vice versa. The in vitro synthesized mutants carried mutations (i) within Walker A and B motifs, (ii) within stretches that are homologous to GTP binding proteins and (iii) which are known to activate GTP binding proteins [20]. We examined both abilities of the CFTR mutants, (i) to generate a whole cell Cl^- conductance upon stimulation with IBMX and forskolin and (ii) to downregulate ENaC. Whole cell conductances generated by each mutant were compared with that of wtCFTR and are therefore expressed as % of G_{wtCFTR} (Fig. 4A, gray bars). In this series of experiments, G_{wtCFTR} was 56 ± 6.3 μS ($n = 9$). wtCFTR inhibited amiloride sensitive Na^+ conductance from 24.2 ± 3.4 μS to 3.4 ± 0.5 μS ($n = 9$). Amiloride sensitive Na^+ conductances were detected in all batches of oocytes expressing the various CFTR mutants, varying between 16 and 41 μS. In order to be able to better compare the inhibitory effects of the various CFTR mutants on G_{Amil}, the fractional inhibition of G_{Amil} is shown in Fig. 4 rather than the absolute amount of Na^+ conductance inhibited by CFTR. It is shown that all mutations affected the CFTR Cl^- channel function, due to reduced expression (e.g. ΔF508) or defective function (e.g. G551D) of the mutant protein. The CFTR mutants K646A, R487Q, G551D, D572N and K615A did not generate significant CFTR Cl^- conductances. These mutants also lost their ability to inhibit ENaC (shown as % inhibition of the initial amiloride sensitive Na^+ conductance, black bars). The mutants S466L, KH483/484AA, R516A and KK611/612AA demonstrated a residual Cl^- channel function, which was paralleled by a limited ability to downregulate ENaC. The Cl^- conductance generated by these mutants was inhibited by gluconate and demonstrated a halide permeability sequence of $Cl^- \geq Br^- > I^-$ (data not shown). These results indicate that CFTR's ability to generate a Cl^- conductance and to inhibit ENaC are closely related and suggest that both properties cannot be separated by introducing mutations into NBF1.

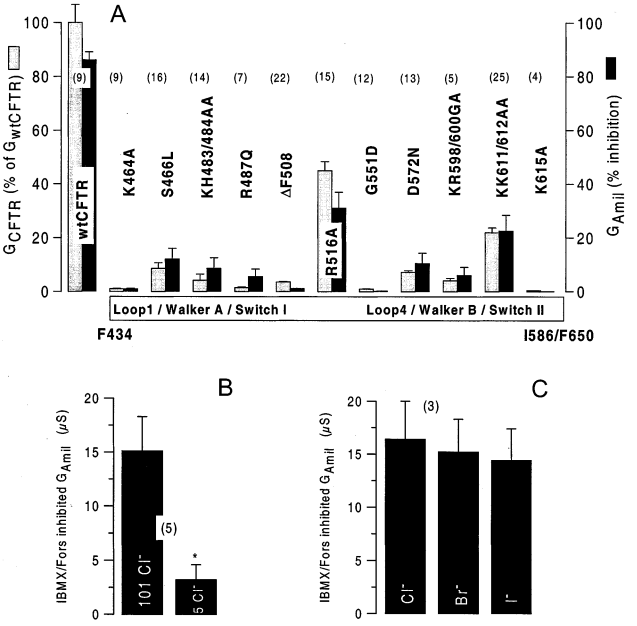

Fig. 4. (A) Impact of mutations located in NBF1 on the activation of whole cell Cl^- conductance (G_{CFTR}) and CFTR dependent inhibition of ENaC (G_{Amil}). Activation of Cl^- conductances in *Xenopus* oocytes expressing wtCFTR and mutant CFTR (expressed as % of G_{wtCFTR}, gray bars) and inhibition of amiloride sensitive ENaC conductance (expressed as % inhibition = $G_{Amil-con} - G_{Amil-IBMX/Fors}/G_{Amil-con}$, black bars). Figures in parentheses indicate numbers of experiments. (B) Summary of amiloride sensitive Na^+ conductance (ENaC) inhibited during activation of coexpressed CFTR. Inhibition of ENaC is significantly stronger in the presence of high Cl^- (101 mmol/l) compared to low extracellular Cl^- (5 mmol). (C) Inhibition of amiloride sensitive ENaC conductance by CFTR was similar with either Cl^-, Br^- or I^- present in the extracellular bath solution. Stimulation of CFTR whole cell Cl^- conductance was significant under all experimental conditions. Figures in parentheses indicate numbers of experiments.

3.3. Halides conducted by CFTR inhibit ENaC

The whole cell conductance activated during stimulation of CFTR was reduced from 43 ± 5.6 down to 19 ± 3.2. μS ($n = 5$), when extracellular Cl^- was re-

placed by gluconate. G_{ENaC}, inhibited upon stimulation of CFTR with IBMX and forskolin, was significantly reduced when CFTR was stimulated in the presence of low (5 mmol/l) extracellular Cl^- (Fig. 4B). This confirms previous results, demonstrating that inhibition of ENaC by CFTR largely depends on the CFTR Cl^- current [18]. In addition, we examined whether the inhibition of ENaC depends on the halide present in the extracellular bath solution. When bath Cl^- was replaced by either Br^- or I^-, a halide permeability sequence for the cAMP activated Cl^- conductance of $Cl^- \geq Br^- > I^-$ was obtained, similar to what has been published previously [13]. CFTR inhibited ENaC in the presence of either Cl^-, Br^- or I^- in the extracellular bath solution (Fig. 4C). Inhibition of ENaC by CFTR varied only slightly but not significantly with the various halides present in the bath solution. Taken together, these data suggest that inhibition of ENaC by CFTR relies on the anion current generated by CFTR and is largely in the presence of the impermeable anion gluconate.

4. Discussion

4.1. No evidence for the role of PDZ domain proteins during activation of CFTR in Xenopus oocytes

NHERF1 and NHERF2 are cytoplasmic phosphoproteins which are involved in PKA dependent regulation of the Na^+/H^+ antiporter NHE3 [21]. NHERF contains common sites for protein interaction, called PDZ domains [12]. A domain complementary to these PDZ domains has been identified at the C-terminal end of CFTR and binding of NHERF1 and NHERF2 to CFTR has been shown in subsequent experiments [6,9,11,12]. In airway epithelial cells, CFTR binds to the recently identified NERHF homologue EBP50, thereby anchoring CFTR to the cytoskeleton via binding to ezrin [9]. However, coexpression of β2-adrenergic receptors, E3KARP and ezrin together with CFTR only slightly augmented activation of CFTR by isoproterenol [11]. Another study demonstrated PDZ interaction of CFTR with cytoskeletal elements being essential for polarized distribution of CFTR in airway and kidney epithelial cells [7]. Because ezrin may serve as an PKA anchoring protein, it has been suggested that interaction of NHERF (EBP50) with CFTR and ezrin localizes PKA in close proximity to CFTR and thereby promotes phosphorylation of the R domain. In the present experiments we were not able to demonstrate the requirement of the PDZ-BD for the activation of CFTR in *Xenopus* oocytes. Although the L1480V-CFTR mutant allows only marginal binding of NHERF to CFTR [12], activation of L1480V-CFTR by IBMX and forskolin was comparable to that of wtCFTR and even the N-terminal half of CFTR (E831X) was still partially activated by increase in intracellular cAMP. It was a surprising and rather unexpected result that E1474X-CFTR caused a very large Cl^- conductance which was significantly enhanced compared to wtCFTR. Whether this is due to enhanced maturation of the CFTR protein or due to a change of the regulatory properties of CFTR remains currently unknown. Moreover, coexpression of CFTR with wt or mutant NHERF1 or NHERF2 did not affect activation of CFTR. Therefore, our results do not support a role of CFTR's PDZ-BD or NHERF in PKA dependent regulation of CFTR in *Xenopus* oocytes.

4.2. No evidence for the role of the CFTR PDZ-BD in the inhibition of ENaC in Xenopus oocytes

The present experiments were undertaken in order to examine the role of the CFTR PDZ-BD in the downregulation of ENaC which has been described extensively [13,14]. Lack of inhibition of ENaC by mutant CFTR is probably the reason for enhanced electrolyte absorption in cystic fibrosis [1,14]. CFTR binds to the scaffolding protein EBP50 and is colocalized with other proteins such as YAP65 and the non-receptor tyrosine kinase c-Yes. These proteins may control epithelial Na^+ transport [22]. A model appears attractive in which CFTR inhibits ENaC with the help of PDZ domain proteins, YAP65 and non-receptor kinases. The present results, however, do not support the idea of a CFTR/ENaC interaction via PDZ domain proteins, since all mutants lacking the CFTR PDZ-BD are still able to downregulate ENaC upon increase of intracellular cAMP. Moreover, coexpression of NHERF1 or NHERF2 did not affect the downregulation of ENaC by CFTR. Taken together, the present experiments show that inhibition of ENaC by CFTR in *Xenopus*

oocytes does not occur via the C-terminal PDZ binding domain.

4.3. Cl^- transport and inhibition of ENaC are intimately related

Previous work demonstrated that acute downregulation of ENaC by CFTR requires an intact NBF1 and also depends on the Cl^- transport activated during stimulation of CFTR [15]. This provokes the question whether there is a specific site within NBF1 that is responsible for inhibition of ENaC or whether it is the CFTR Cl^- conductance that is crucial for the inhibition. However, activation of CFTR Cl^- conductance requires a functional NBF1. We found in a previous study that CFTR truncations comprising NBF1 are able to inhibit ENaC slightly albeit significantly. Some of these truncations did not activate an additional Cl^- conductance upon stimulation with IBMX/forskolin but induced an enhanced baseline Cl^- conductance in these oocytes [15]. These results and the present data suggest that the ability of NBF1 containing CFTR truncations to inhibit ENaC is largely augmented by a Cl^- current [15]. We therefore introduced mutations into NBF1 sites which are essential for binding/hydrolysis of ATP and GTP and which have homology to GTP binding proteins such as Walker A (loop L1) (K464A, S466L), switch I motif (KH483/484AA, R487A), switch II motif (loop L4, G551D) and Walker B (D572N) [23]. Furthermore, NBF1 sequences which could potentially serve as activator sequences for G proteins were mutated (KR598/600GA, KK611/612AA, K615A). The region between K598 and K615 contains two basic residues at the N-terminal side and the sequence B-B-X-X-B at the C-terminus, which is similar to structural determinants for the G_i stimulating function of the active insulin-like growth factor II receptor sequence [24]. All mutations examined here caused a reduced or even a complete loss of the Cl^- conductance. To the same degree, the ability of these mutants to downregulate ENaC was reduced or completely lost, very similar to what has been described previously [18]. We never observed an independent change of both CFTR properties. It suggests that Cl^- transport is crucial for the inhibition of ENaC. This is further confirmed by the Cl^- replacement experiments shown in Fig. 4B. However, in contrast to the CFTR controlled release of ATP, inhibition of ENaC is largely independent of the halide present in the bath solution [16].

How does the CFTR Cl^- conductance modulate the activity of epithelial Na^+ channels? As one possibility, the intracellular Cl^- concentration could change and could directly affect ENaC channel activity [25]. However, assuming similar changes in the intracellular Cl^- concentration occur in the sweat duct epithelium during stimulation of CFTR, a direct inhibition of ENaC by intracellular Cl^- appears unlikely. It has been shown for the sweat duct epithelium that activation of CFTR is paralleled by an increase in ENaC conductance [5]. This suggests a contribution of additional proteins, expressed differentially in airways, colon and sweat duct. As another possibility, the intracellular Cl^- concentration may increase in airways and intestinal epithelium during stimulation of CFTR but may fall in the sweat duct epithelium. The currently available data are limited and do not support large changes in intracellular Cl^- concentration during activation of CFTR in sweat duct or airway/colonic epithelium [26–28]. Here, more detailed analysis of the intracellular Cl^- concentration during activation of ion transport in all three tissues will be required. Interestingly and in contrast to airways and colon, CFTR is expressed in both luminal and basolateral membrane of the sweat duct epithelium and seems predominantly activated in basolateral membrane [26,29,30]. Cytosolic Cl^- has been reported previously to regulate amiloride sensitive Na^+ channels in mouse salivary duct cells via Cl^- sensitive trimeric G proteins [31]. However, in unpublished experiments performed in *Xenopus* oocytes, we did not find any contribution of GTP binding proteins or other Cl^- sensitive proteins, such as nucleoside diphosphate kinase to the regulation of ENaC by CFTR [32]. Future studies will have to demonstrate the link between Cl^- conductance/concentration and regulation of ENaC.

Acknowledgements

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