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Topical Review

CFTR, A Regulator of Channels

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Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated Cl^- channel that is defective in cystic fibrosis. This statement is found in most of the articles somehow dealing with CFTR. However, the initial characterization of this puzzling protein as a conductance regulator is probably more appropriate. As we have learned over the past few years, CFTR controls the function of various other membrane conductances and the list of putative interactions of CFTR with other channels and cellular functions is continuously growing. Although it has been demonstrated in many cell types that CFTR forms a Cl^- channel, we may speculate that, at least in some tissues, CFTR rather acts through regulation of other membrane conductances or cellular functions. The recent findings on CFTR dependent regulation of other ion channels will challenge scientists to search for the mechanisms underlying the interaction between CFTR and other membrane proteins. In that respect, identification of consensus sites known to be important for protein interaction and isolation of putative cofactors involved in this process will be essential in order to uncover complex signaling cascades. The effects of CFTR on various cellular functions create a rather complicated pathophysiological scenario for the inherited disease cystic fibrosis. It is discussed that in addition to the well described defect of cAMP dependent CFTR Cl^- conductance, several other membrane conductances are prone to participate in the impaired epithelial electrolyte transport in cystic fibrosis.

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Key words: CFTR — Cystic fibrosis — Epithelial Na^+ conductance — ENaC — AQP3 — K_VLQT-1

Contribution of CFTR to the Enhanced Na^+ Conductance in Cystic Fibrosis

Enhanced amiloride inhibitable short-circuit currents and thus enhanced Na^+ reabsorption has been detected in the airways of cystic fibrosis patients more than 10 years ago [8, 9]. Additional evidence for an enhanced Na^+ conductance in airway cells came from patch-clamp studies on primary cultures of CF and non-CF airway epithelial cells [58]. Recent data were obtained in very elegant studies on *Xenocraft* models and so called "biofibers" showing both enhanced NaCl and fluid reabsorption in CF airway epithelia [120]. According to in vivo measurements of the rectal potential difference enhanced Na^+ conductance was suggested to take place in the intestinal epithelium of CF patients [83]. These findings were supported by subsequent studies in CFTR (*-1-*) knockout mice [37]. Ussing chamber experiments performed very recently on human CF and non-CF mucosal biopsies demonstrated enhanced Na^+ conductance in apical membranes of intestinal mucosa of CF patients [73]. Epithelial Na^+ channels expressed in apical membranes of reabsorptive epithelial cells were cloned initially from rat colon [30]. Interestingly, ENaC and CFTR are colocalized in many tissues e.g., the kidney collecting duct, the colonic epithelium, the airways and several others [58]. It should be mentioned, however, that for some epithelial tissues the physiological significance of CFTR expression remains obscure. Thus, the kidney function does not seem to be affected in CF.

Coexpression of both CFTR and ENaC in MDCK cells or NIH 3T3 fibroblasts demonstrated inhibition of

amiloride-sensitive Na^+ short-circuit currents and a reduction of whole Na^+ conductances upon activation of CFTR [104]. Coexpression of both proteins in *Xenopus* oocytes also showed acute and reversible inhibition of ENaC by activation of CFTR [69]. However, ENaC was not inhibited by mutant ΔF508-CFTR, suggesting that enhanced Na^+ conductance in cystic fibrosis is caused by a lack of downregulation of ENaC by defective CFTR. Subsequent studies identified the phenomenon of CFTR-dependent inhibition of ENaC also in other cell types like cultured mammalian kidney and colonic epithelial cells [26, 63]. Experiments performed on planar lipid bilayers and excised membrane patches suggested a rather direct mechanism for the inhibition of ENaC by CFTR [45, 105]. In addition, actin filaments seem to augment the capability of CFTR to downregulate ENaC [47]. Apparently, coexpression of CFTR inverts protein kinase A-mediated regulation of epithelial Na^+ channels by interfering with the gating mechanism of the channel, thus turning activation of ENaC by protein kinase A into channel inhibition [105]. Some hints for direct protein interaction of α-rENaC with cytosolic domains of CFTR came from two-hybrid analysis in yeast [60]. On the other hand, recent studies show that Cl^- flux through CFTR is important for the downregulation of ENaC [12]. Inhibition of ENaC by CFTR was also found in rat colonic epithelial cells [26] and in normal human but not in CF airways [71]. Thus, epithelial cells, which might have reabsorptive function under resting conditions, switch to secretion when stimulated with secretagogues enhancing intracellular cAMP [33, 34] (Fig. 1).

Such a switch does not take place in cystic fibrosis airway epithelial cells. Therefore, the superficial epithelium is hyperabsorbing NaCl due to enhanced Na^+ conductance. On the other hand, electrolyte secretion, which occurs mainly in the submucosal glands, is largely inhibited due to the lack of CFTR Cl^- conductance [27]. Hyperabsorption will dehydrate the airways resulting in the well described pathophysiological consequences of enhanced mucus viscosity, decreased mucociliary clearance and bacterial colonization of the airways. It should be mentioned that bacterial infections of CF airways were also explained by other mechanisms based on defective clearance of *Pseudomonas aeruginosa* due to impaired bacterial killing by defensines [102].

Does CFTR Control Outwardly Rectifying (ICOR) Cl^- Channels?

Outwardly rectifying Cl^- channels of about 50 pS single channel conductance (ICOR) were detected frequently in excised membrane patches of epithelial cells [39, 114]. Because this channel is so *abundant*, it was assumed to form the apical conductive pathway for Cl^- secretion that is defective in cystic fibrosis [64, 93]. Others, however, were unable to activate the channel by stimulation of the cAMP pathway and found the channel only occasionally in cell-attached patches, suggesting a cytosolic inhibitory molecule that keeps the channel closed in the intact cell [54, 55].

According to more recent studies, expression and activation of CFTR induces a typical CFTR Cl^- conductance and in parallel activates outwardly rectifying Cl^- currents [29]. The incidence of ICOR channels has been claimed to be enhanced in the presence of CFTR [49]. According to a recent model ICOR is coactivated with CFTR by an autocrine mechanism based on the finding that CFTR, when activated by protein kinase A, does not only conduct Cl^- but also ATP [49, 97]. ATP will be secreted to the apical side and bind to purinergic receptors which are probably coupled to ICOR channels via G-proteins thus activating outwardly rectifying Cl^- currents [46]. However, before such a model can finally be accepted some essential unresolved issues have to be settled like that of the low channel incidence in intact epithelial cells [54]; the uncertainties regarding the ATP permeability of CFTR [89]; and the low efficacy of rather potent blockers of ICOR in intact epithelial cells [32].

CFTR's impact on Ca^{2+} and Swelling-Activated Cl^- Conductances

Previous studies in rather nondifferentiated cultured epithelial cells detected Ca^{2+}-activated Cl^- currents that were augmented by expression of CFTR [56]. Moreover, inactivation of transient Ca^{2+}-dependent Cl^- currents was delayed when CFTR was activated [2]. This phenomenon was not observed in CF epithelial cells. However, in the native colonic tissue a luminal Ca^{2+}-activated Cl^- conductance has thus far not been observed and CFTR seems to form the only luminal Cl^- conductance [72]. Moreover, regulation of Ca^{2+}-activated Cl^- conductances by CFTR was somehow different in *Xenopus* oocytes. Here, the amplitude of Ca^{2+}-activated Cl^- currents was attenuated during activation of CFTR [61]. At any rate, these and other studies [77] suggest that CFTR somehow controls the activity of the Ca^{2+}-activated Cl^- currents. Whether Ca^{2+}-activated Cl^- currents are augmented in intestinal, respiratory and pancreatic duct epithelia of CFTR (*-1-*) knockout mice is still a matter of debate [36, 116, 117]. Although the molecular structure of the swelling-activated Cl^- channel remains obscure [25, 109] it has been shown that expression of CFTR controls the amplitude of swelling-activated Cl^- currents in cultured colonic carcinoma cells [56, 57]. In another study, the volume activated Cl^- conductance was significantly reduced in CFTR (*-1-*) knockout mice when compared to non-CF control mice [110]. Although poorly understood, these results point to the fact that CFTR also controls the activity of other Cl^- channels

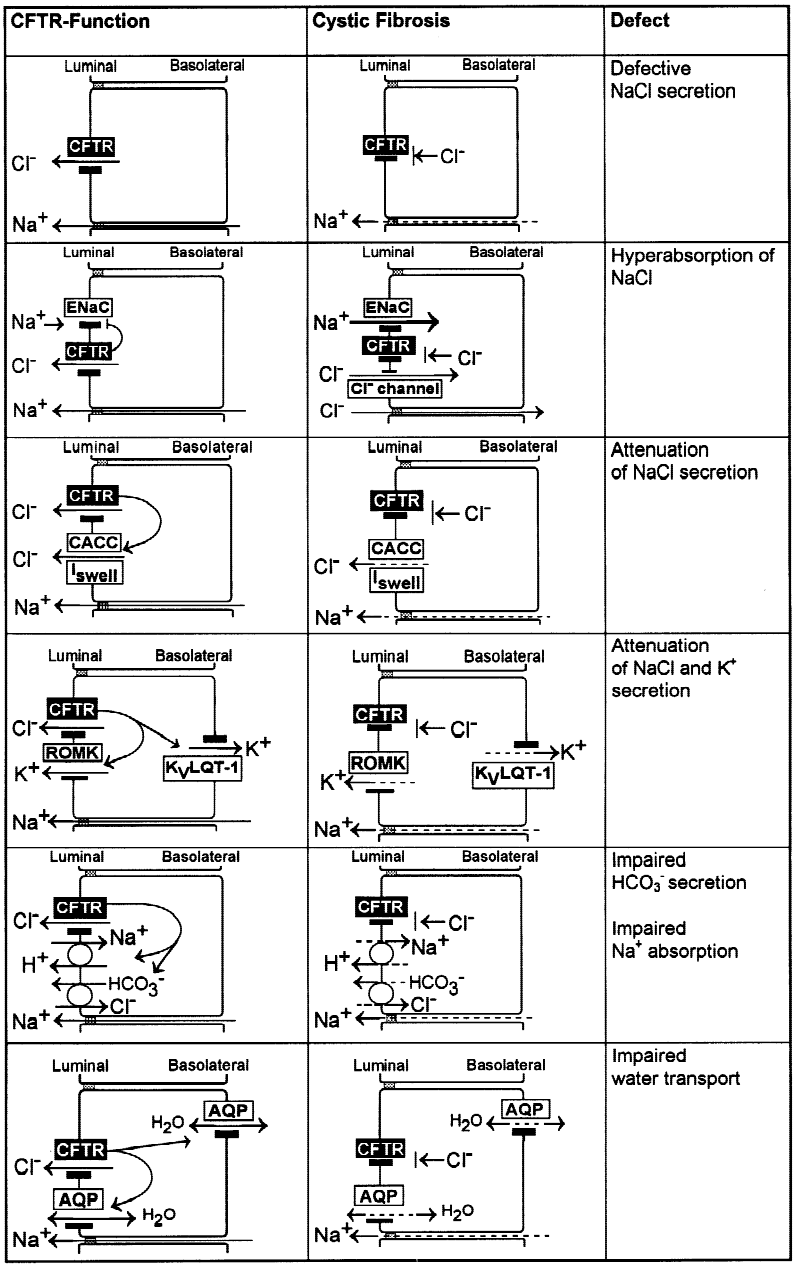

Fig. 1. Possible impact of CFTR on membrane proteins and its hypothetical role in epithelial ion transport. The table summarizes putative functions of CFTR under physiological conditions and defects of epithelial transport when CFTR is defective as in cystic fibrosis. (ENaC = epithelial Na^+ channel; Cl^- channel = non-CFTR Cl^- channel; CACC = Ca^{2+}-activated Cl^- channel; ICOR = intermediate conductance outwardly rectifying Cl^- channel; I_{swell} = Cl^- channel activated by cell swelling; AQP = aquaporin, ROMK = renal K^+ channel; K_VLQT-1 = voltage-dependent delayed rectifier K^+ channel).

(Fig. 1). Therefore, Cl^- channels of single channel amplitude smaller than the typical 7 pS were found to be regulated by CFTR in some epithelia [3, 28, 59, 119].

Regulation of K_VLQT-1 K^+ Channels by CFTR?

Basolateral K^+ conductance is essential to maintain electrolyte secretion and is coactivated during stimulation of epithelial cells by agonists enhancing intracellular cAMP [113]. Previous studies showed that cAMP activated K^+ conductance is present in CFPAC-1 cells expressing functional CFTR but not in the parental cell line [67]. However, in another study using the same cell line a K^+ conductance was activated by cAMP in the absence of CFTR [112]. When CFTR was expressed in *Xenopus* oocytes and activated by intracellular cAMP, an enhanced K^+ conductance was detected [70]. This was not observed when ΔF508-CFTR was expressed. Strikingly, the CFTR-activated K^+ conductance detected in *Xenopus* oocytes could be blocked by the cromanol 293B, which blocks cAMP-activated K^+ conductance very potently in colonic epithelial cells, suggesting that the same type of K^+ conductance is expressed in both *Xenopus* oocytes and colonic epithelial cells [51, 66]. More recent studies

demonstrated that cAMP-activated K^+ channels expressed in colonic epithelial cells and *Xenopus* oocytes are homologues of K_VLQT-1. This K^+ channel was cloned originally from mouse heart and identified subsequently also from human and *Xenopus* tissues [5, 91]. K_VLQT-1 forms K^+ channels probably as a heterocomplex with IsK (minK) and is inhibited by the new class of cromanol compounds [13]. K_VLQT-1 channels cloned from mouse and human heart contain N-terminal phosphorylation sites for protein kinase A and are activated by an increase of intracellular cAMP when expressed in *Xenopus* oocytes (*unpublished results* from the authors laboratory). So far, we could not demonstrate CFTR-dependent activation of human and mouse K_VLQT-1 when coexpressed in *Xenopus* oocytes (*unpublished results* from the author's laboratory). It has to be examined in subsequent studies whether CFTR-dependent activation of K_VLQT-1 K^+ currents can be detected in native epithelial cells and whether specific isoforms of K_VLQT-1 are expressed in this tissue [19, 20] (Fig. 1).

CFTR Enhances the Affinity of K^+ Channels for Sulfonylurea Compounds

ATP-sensitive K^+ channels exists as multimeres consisting of K^+ channel subunits and sulfonylurea receptors (SUR) [1]. In the absence of SUR sulfonylurea compounds are rather ineffective in blocking ATP-sensitive K^+ channels. Enhanced sensitivity of renal K^+ channels ROMK2 (Kir1.2) as well as Kir1.1a and Kir6.1 for sulfonylurea compounds and an increase in the open probability of these channels was reported when CFTR was coexpressed in *Xenopus* oocytes [44, 75, 90]. In the absence of either CFTR or SUR, ROMK2 is hardly blocked by glibenclamide when expressed in oocytes. Like CFTR, SUR belongs to the family of ABC transporters. The experiments described above indicate that SUR obviously can be replaced by CFTR with respect to its function as a receptor for sulfonylurea compounds. Alternatively, CFTR may modify the binding affinity of ROMK2 for glibenclamide. In a subsequent report by the same group, the first nucleotide binding fold (NBF1) of CFTR was demonstrated as being essential for the enhanced sensitivity of ROMK2 towards glibenclamide [76]. Currently, it is poorly understood how CFTR interacts with these K^+ channels and what mechanism is responsible for the enhanced glibenclamide sensitivity.

Impact of CFTR on the Regulation of Intracellular pH

Several reports indicate CFTR's permeability for HCO_3^- in cultured and native intestinal epithelial cells [43, 86, 98]. Thus, cytosolic as well as extracellular pH might be altered by CFTR. In fact, acidification of cytosolic pH was found to be dependent on CFTR, although no differences in resting cytosolic pH in normal and CF airway cells could be detected [31, 86, 115]. In the pancreatic duct, CFTR's impact on HCO_3^- secretion is probably more indirect, by serving as a recycling pathway for Cl^- during HCO_3^- transport by the HCO_3^-/Cl^- antiporter rather than functioning as a HCO_3^- channel [53, 81]. In CF, a lack of sufficient alkalization and stasis of the pancreatic juice leads to enzyme activation and tissue destruction [53].

CFTR is also present in intracellular membranes like endosomal vesicles [11, 68]. Endosomal CFTR may serve as a parallel Cl^- conductance counterbalancing the activity and charge movement of the H^+-ATPase, which acidifies the endosomal compartment [4]. In cystic fibrosis, acidification of the endosomal compartment should be impaired. In fact, a higher endosomal pH was found in CF-epithelial cells at least in one study [4, 99]. The performance of enzymes participating in protein sialylation in the endoplasmic reticulum is largely pH dependent. Accordingly, decreased sialylation and enhanced sulfation of glycoproteins was detected in CF cells [4, 15, 24]. Oversulfation and overfucosylation of glycoproteins leads to enhanced attachment of pathogenic bacteria like *Pseudomonas aeruginosa* [18]. This mechanism very likely contributes to the high morbidity and mortality seen in CF after onset of chronic infections of the airways by *P. aeruginosa* [15]. However, it should be noticed that attachment of *P. aeruginosa* in CF-airways was explained by another mechanisms that proposes CFTR as a receptor for *P. aeruginosa* in the airways [85].

The Na^+/H^+ exchanger contributes to the regulation of cytosolic pH. In more recent studies it was found that electroneutral absorption of NaCl in the small intestine occurs via parallel Na^+/H^+ and Cl^-/HCO_3^- exchangers. This process was shown to be inhibited by cAMP in normal tissues from control mice, but not in tissues from CFTR(*-1-*) knockout mice [16]. Defective regulation of either of the above two antiporters by mutant CFTR in CF obviously affects absorption of NaCl in epithelial tissues and is likely to interfere with intracellular pH homeostasis [86] (Fig. 1).

CFTR Activates a Water Permeability

The CFTR channel pore can accommodate not only anions but also small solutes and water [38, 65]. One study performed in *Xenopus* oocytes suggested that CFTR is able to form a multifunctional aqueous channel essentially participating in transepithelial ion and water transport [38]. Enhanced osmotic water permeability in *Xenopus* oocytes by expression of wtCFTR but not mutant CFTR was confirmed by another group [94]. However, the authors of the later study presented evidence

that H_2O does not move through the CFTR Cl^- channel but rather through a separate H_2O conductance that is identical to an aquaporin water channel that is activated by CFTR. Whether the CFTR-activated water conductance is relevant for mammalian cells, particularly for epithelial cells, is currently under investigation. In fact, very recent studies indicate that CFTR-activated osmotic water permeability is present in normal airway epithelial cells, while this cannot be detected in airway cells from CF patients [95, 96]. The physiological significance of the CFTR-related increase in osmotic water permeability is not clear currently. Activation of a water permeability by CFTR in parallel with the activation of NaCl transport would facilitate both epithelial secretion or absorption (Fig. 1). Thus, defective CFTR–dependent regulation of water permeability is likely to affect electrolyte transport in the airways and it is also likely to contribute to dehydration and changes in rheologic properties of the mucus in CF airways.

How Does CFTR Regulate Other Channels?

It is rather difficult to understand how CFTR is able to affect such a variety of different cellular processes and ion conductances. Apart from CFTR-dependent regulation of the epithelial Na^+ conductance there is currently not much known about these mechanisms. It has been shown that CFTR is able to interfere with cellular exocytosis and endocytosis, thus regulating membrane turnover [7, 10, 106]. Whether CFTR itself is exocytosed and subsequently endocytosed during the process electrolyte secretion is an open question and the answer may depend on the cell type examined [35, 41, 62, 78, 92, 106]. Physical interaction of CFTR with syntaxin 1A, a protein known to be involved in neurosecretion, gives further evidence for the impact of CFTR on membrane turnover [80]. The effects of CFTR seem to be limited to clathrin-dependent endocytosis and do not affect fluid phase endocytosis [68, 103]. The effects of CFTR on other membrane conductances could be due to regulation of exocytosis or endocytosis of the respective membrane proteins. In fact, ENaC has been demonstrated to be regulated by clathrin-mediated endocytosis [100]. However, recent studies show that ENaC consisting of subunits lacking the C-terminal PY motif and thus demonstrating defective ubiquitin-dependent endocytosis are still inhibited by CFTR. This suggests another mechanism than that of CFTR-dependent endocytosis of ENaC [40]. On the other hand, membrane turnover will be paralleled by depolymerization of actin which could influence the activity of other ion channels [42, 87]. In fact, for the CFTR-dependent regulation of ENaC a modulatory action of short actin filaments has already been postulated [6, 47].

Some evidence exists for direct interaction of ENaC and CFTR that came from data obtained by the two-hybrid system [60] and experiments with reconstituted proteins [45]. However, it seems very likely that additional proteins are involved in the interaction of CFTR with other membrane proteins. Within the CFTR protein, several sequences exist that would allow for interaction with other proteins. Thus, the C-terminal end of CFTR contains a sequence that specifically binds to a conserved motif for protein interaction, the PDZ1 domain of NHERF, a regulatory factor of the Na^+/H^+ exchanger type 3 (NHE3) [111]. NHERF belongs to a class of proteins containing PDZ–domains that facilitate protein interaction. This interaction is essential for the control of different cellular events like cAMP-dependent regulation of the epithelial brush border Na^+/H^+ exchanger, activation of a tyrosine kinase and stimulation of the Na-P_i cotransport in kidney cortex during dietary P_i restriction [17, 118]. In fact, it has been demonstrated recently that CFTR interacts via a C-terminal stretch of four amino acids (DTRL) with cytoskeletal proteins and thereby may influence the activity of other channels as well [101].

Other good candidates for mediating CFTR's regulatory effects on epithelial membrane conductances would be GTP-binding proteins. The nucleotide-binding domains of CFTR were shown to hydrolyze ATP as well as GTP [88]. CFTR contains several stretches of sequences known to activate GTP-binding proteins [21, 82]. In addition, a G protein-like sequences has been identified within CFTR [14, 74]. Apart from two studies, involvement of G proteins in CFTR dependent regulation of other membrane conductances has not been examined in detail. G proteins were shown to essentially participate in CFTR-dependent regulation of ICOR [46]. Other studies demonstrate activation of a subtype of inhibitory G proteins by increase of intracellular Cl^- that inhibits epithelial Na^+ currents in mandibular duct cells [22, 52]. A very similar mechanism has been described by the same group for the so-called Na^+ feedback, known to be present in absorptive epithelia [23, 52, 84]. However, CFTR-dependent inhibition of ENaC in *Xenopus* oocytes was not strictly dependent on the absolute intracellular Cl^- concentration in another report [12]. Interestingly, another class of proteins, the nucleoside diphosphate kinases were demonstrated to be regulated by the intracellular Cl^- concentration [79, 108]. Nucleoside diphosphate kinases transfer terminal phosphates from ATP to GDP, thus facilitating the generation of GTP and the activation of GTP-dependent intracellular processes. It was shown that nucleoside diphosphate kinases are expressed in the airway epithelium where they participate in the control of ion secretion [108]. In this respect it might be worth mentioning that the epithelial Na^+ channel has been demonstrated to be inhibited upon accumulation of intracellular cGMP [48, 50].

Conclusions

Properties of CFTR at the single channel level have been well established. There is now growing evidence that in addition to its function as a Cl^- channel it may act as a conductance regulator. In that sense, the initial characterization of CFTR as a channel regulator seems fully appropriate. Coordination of the proteins contributing to epithelial ion transport could be the essential task of CFTR in epithelial cells. In colon and airways both CFTR and ENaC are colocalized in epithelial cells [26, 58, 107]. These proteins are known to participate in secretion (CFTR) and absorption (ENaC) of electrolytes. Coordinate regulation of these conductances, i.e., turning on CFTR and switching off ENaC during induction of ion secretion is enabled by CFTR [34]. Very little is known about the functional consequences of the other membrane conductances regulated by CFTR. However, failure in CFTR-dependent regulation of these proteins is likely to contribute to the defects observed in CF. Recent experiments have just started to uncover the mechanisms underlying the CFTR-dependent control of other transport proteins. There is no doubt that future studies will identify additional functional links to CFTR and unmask novel mechanisms of membrane protein interaction. Ultimately, this should lead to an improved understanding of the polymorphic disease cystic fibrosis.

References

1. Aguilar-Bryan, L., Clement, J.P., 4th, Gonzalez, G., Kunjilwar, K., Babenko, A., Bryan, J. 1998. *Physiol. Rev.* **78:**227–245
2. Allert, N., Leipziger, J., Greger, R. 1992. *Pfluegers Arch.* **421:**403–405
3. Argent, B.E., Gray, M.A., Greenwell, J.R. 1987. *J. Physiol.* **391:**33P
4. Barasch, J., Kiss, B., Prince, A., Saiman, L., Gruenert, D.C., Al-Awqati, Q. 1991. *Nature* **352:**70–73
5. Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., Romey, G. 1996. *Nature* **384:**78–80
6. Berdiev, B.K., Prat, A.G., Cantiello, H.F., Ausiello, D.A., Fuller, C.M., Jovov, B., Benos, D.J., Ismailov, I.I. 1996. *J. Biol. Chem.* **271:**17704–17710
7. Biwersi, J., Emans, N., Verkman, A.S. 1996. *Proc. Natl. Acad. Sci. USA* **93:**12484–12489
8. Boucher, R.C., Stutts, M.J., Knowles, M.R., Cantley, L., Gatzy, J.T. 1986. *J. Clin Invest.* **78:**1245–1252
9. Boucher, R.C., Cotton, C.U., Gatzy, J.T., Knowles, M.R., Yankaskas, J.R. 1988. *J. Physiol.* **405:**77–103
10. Bradbury, N.A., Jilling, T., Gabor, B., Sorscher, E.J., Bridges, R.J., Kirk, K.L. 1992. *Science* **256:**530–531
11. Bradbury, N.A., Cohn. J.A., Venglarik, C.J., Bridges, R.J. 1994. *J. Biol. Chem.* **269:**8296–8302
12. Briel, M., Greger, R., Kunzelmann, K. 1998. *J. Physiol.* **508.3:**825–836
13. Busch, A.E., Busch, G.L., Ford, E., Suessbrich, H., Lang, H.J., Greger, R., Kunzelmann, K., Attali, B., Stühmer, W. 1997. *Br. J. Pharmacol.* **122:**187–189
14. Carson, M.R., Welsh, M.J. 1995. *Biophys. J.* **69:**2443–2448
15. Cheng, P.W., Boat, T.F., Cranfill, K., Yankaskas, J.R., Boucher, R.C. 1989. *J. Clin. Invest.* **84:**68–72
16. Clarke, L.L., Harline, M.C. 1996. *Am. J. Physiol.* **270:**G259–67
17. Custer, M., Spindler, B., Verrey, F., Murer, H., Biber, J. 1997. *Am. J. Physiol.* **273:**F801–6
18. Davies. J.D., Stern, M., Dewar, A., Caplen, N.J., Munkonge, F.M., Pitt, T., Sorgi, F., Huang, L., Bush, A., Geddes, D.M., Alton, E.W. 1997. *Am. J. Respir. Cell. Mol. Biol.* **16:**657–663
19. Demolombe, S., Mohammad-Panah, R., Barbry, P., Barhanin, J., Kunzelmann, K., Baró, I., Escande, D. 1997. *11th North American CF Conference, Nashville* 23. - 26. 10. 97 (*Abstr.*)
20. Demolombe, S., Baro, I., Pereon, Y., Bliek, J., Mohammad-Panah, R., Pollard, H., Morid, S., Mannens, M., Wilde, A., Barhanin, J., Charpentier, F., Escande, D. 1998. *J. Biol. Chem.* **273:**6837–6843
21. Diamond, G., Scanlin, T.F., Zasloff, M.A., Bevins, C.L. 1991. *J. Biol. Chem.* **266:**22761–22769
22. Dinudom, A., Young, J.A., Cook, D.I. 1993. *J. Membrane Biol.* **135:**289–295
23. Dinudom, A., Harvey, K.F., Komwatana, P., Young, J.A., Kumar, S., Cook, D.I. 1998. *Proc. Natl. Acad. Sci. USA* **95:**7169–7173
24. Dosanjh, A., Lencer, W., Brown, D., Ausiello, D.A., Stow, J.L. *Am. J. Physiol.* **266:**C360–6
25. Duan, D., Winter, C., Cowley, S., Hume, J.R., Horowitz, B. 1997. *Nature* **390:**417–421
26. Ecke, D., Bleich, M., Greger, R. 1996. *Pfluegers Arch.* **431:**984–986
27. Engelhardt, J.F., Yankaskas, J.R., Ernst, S.A., Yang, Y., Marino, C.R., Boucher, R.C., Cohn, J.A., Wilson, J.M. 1992. *Nature genetics* **2:**240–248
28. Fischer, H., Kreusel, K.-M., Illek, B., Bachen, T.E., Hegel, U., Clauss, W. 1992. *Pfluegers Arch.* **422:**159–167
29. Gabriel, S.E., Clarke, L.L., Boucher, R.C., Stutts, M.J. 1993. *Nature* **363:**263–268
30. Garty, H., Palmer, L.G. 1997. *Physiol. Rev.* **77:**359–396
31. Gottlieb, R.A., Dosanjh, A.. 1996. *Proc. Natl. Acad. Sci. USA* **93:**3587–3591
32. Greger, R., Nitschke, R.B., Lohrmann, E., Burhoff, I., Hropot, M., Englert, H.C., Lang, H.J. 1991. *Pfluegers Arch.* **419:**190–196
33. Greger, R., Mall, M., Bleich, M., Ecke, D., Warth, R., Riedemann, N., Kunzelmann, K. 1996. *J. Mol. Med.* **74:**527–534
34. Greger, R., Bleich, M., Leipziger, J., Ecke, D., Mall, M., Kunzelmann, K. 1997. *NIPS* **12:**62–66
35. Greger, R., Thiele, I., Warth, R., Bleich, M. 1998. *Pfluegers Arch.* **436:**538–544
36. Grubb, B.R., Vick, R.N., Boucher, R.C. 1994. *Am. J. Physiol.* **266:**C1478–C1483
37. Grubb, B.R., Boucher, R.C. 1997. *Am. J. Physiol.* **272:**G393–G400
38. Hasegawa, H., Skach, W., Baker, O., Calayag, M.C., Lingappa, V., Verkman, A.S. 1992. *Science* **258:**1477–1479
39. Hayslett, J.P., Gögelein, H., Kunzelmann, K., Greger, R. 1987. *Pfluegers Arch.* **410:**487–494
40. Hopf, A., Mall, M., Greger, R., Kunzelmann, K. 1998. *Pfluegers Arch.* **435:S1:**P11-3 (*Abstr.*)
41. Hug, M.J., Thiele, I.E., Greger, R. 1997. *Pfluegers Arch.* **434:**779–784
42. Hug, T., Koslowsky, T., Ecke, D., Greger, R., Kunzelmann, K. 1995. *Pfluegers Arch.* **429:**682–690
43. Illek, B., Yankaskas, J.R., Machen, T.E. 1997. *Am. J. Physiol.* **272:**L752–51
44. Ishida-Takahashi, A., Otani, H., Takahashi, C., Washizuka, T., Tsuji, K., Noda, M., Horie, M., Sasayama, S. 1998. *J. Physiol.* **508:**23–30

45. Ismailov, I.I., Awayda, M.S., Jovov, B., Berdiev, B.K., Fuller, C.M., Dedman, J.R., Kaetzel, M.A., Benos, D.J. 1996*a. J. Biol. Chem.* **271:**4725–4732
46. Ismailov, I.I., Jovov, B., Fuller, C.M., Berdiev, B.K., Keeton, D.A., Benos, D.J. 1996*b. J. Biol. Chem.* **271:**4776–4780
47. Ismailov, I.I., Berdiev, B.K., Shlyonsky, V.G., Fuller, C.M., Prat, A.G., Jovov, B., Cantiello, H.F., Ausiello, D.A., Benos, D.J. 1997. *Am. J. Physiol.* **272:**C1077–C1086
48. Jain, L., Chen, X.J., Brown, L.A., Eaton, D.C. 1998. *Am. J. Physiol.* **247:**L475–L484
49. Jovov, B., Ismailov, I.I., Benos, D.J. 1995. *J. Biol. Chem.* **270:**1521–1528
50. Kelley, T.J., Cotton, C.U., Drumm, M.L. 1998. *Am. J. Physiol.* **274:**L990–L996
51. Kim, K.-M., Adachi, T., Nielsen, P.J., Terashima, M., Lamers, M.C., Köhler, G., Reth, M. 1994. *EMJO J.* **13:**3793–3800
52. Komwatana, P., Dinudom, A., Young, J.A., Cook, D.I. 1996. *Proc. Natl. Acad. Sci. USA* **93:**8107–8111
53. Kopelman, H., Corey, M., Gaskin, K., Durie, P., Weizman, Z., Forstner, G. 1988. *Gastroenterology*. **95:**349–355
54. Kunzelmann, K., Pavenstädt, H., Greger, R. 1989. *Pfluegers Arch.* **415:**172–182
55. Kunzelmann, K., Tilmann, M., Hansen, C.P., Greger, R. 1991. *Pfluegers Arch.* **418:**479–490
56. Kunzelmann, K., Allert, N., Kubitz, R., Breuer, W.V., Cabantchik, Z.I., Normann, C., Schumann, S., Leipziger, J., Greger, R. 1994*a. Pfluegers Arch.* **428:**76–83
57. Kunzelmann, K., Slotki, I.N., Klein, P., Koslowsky, T., Ausiello, D.A., Greger, R., Cabantchik, Z.I. 1994*b. J. Cell. Physiol.* **161:**393–406
58. Kunzelmann, K., Kathöfer, S., Greger, R. 1995. *Pfluegers Arch.* **431:**1–9
59. Kunzelmann, K., Kathöfer, S., Hipper, A., Gruenert, D.C., Greger, R. 1996. *Pfluegers Arch.* **431:**578–586
60. Kunzelmann, K., Kiser, G., Schreiber, R., Riordan, J.R. 1997*a. FEBS Lett.* **400:**341–344
61. Kunzelmann, K., Mall, M., Briel, M., Hipper, A., Nitschke, R., Ricken, S., Greger, R. 1997*b. Pfluegers Arch.* **434:**178–181
62. Lehrich, R.W., Aller, S.G., Webster, P., Marino, C.R., Forrest, J.N. 1998. *J. Clin. Invest.* **101:**737–745
63. Letz, B., Korbmacher, C. 1997. *Am. J. Physiol.* **272:**C657–C666
64. Li, M., McCann, J.D., Liedtke, C.M., Nairn, A.C., Greengard, P., Welsh, M.J. 1988. *Nature* **331:**358–360
65. Linsdell, P., Hanrahan, J.W. 1998. *J. Gen. Physiol.* **111:**601–614
66. Lohrmann, E., Burhoff, I., Nitschke, R.B., Lang, H.-J., Mania, D., Englert, H.C., Hropot, M., Warth, R., Rohm, M., Bleich, M., Greger, R. 1995. *Pfluegers Arch.* **429:**517–530
67. Loussouarn, G., Demolombe, S., Mohammad-Panah, R., Escande, D., Baró, I. 1996. *Am. J. Physiol.* **271:**C1565–C1573
68. Lukacs, G.L., Segal, G., Kartner, N., Grinstein, S., Zhang, F. 1997. *Biochem. J.* **328:**353–361
69. Mall, M., Hipper, A., Greger, R., Kunzelmann, K. 1996*a. Pfluegers Arch.* **431S:**0–57 (*Abstr.*)
70. Mall, M., Kunzelmann, K., Hipper, A., Busch, A.E., Greger, R. 1996*b. Pfluegers Arch.* **432:**516–522
71. Mall, M., Bleich, M., Greger, R., Schreiber, R., Kunzelmann, K. 1998*a. J. Clin. Invest.* **102:**15–21
72. Mall, M., Bleich, M., Greger, R., Schürlein, M., Kühr, J., Seydewitz, H.H., Brandis, M., Kunzelmann, K. 1998*b. Am. J. Physiol.* (*in press*)
73. Mall, M., Bleich, M., Kühr, J., Brandis, M., Greger, R., Kunzelmann, K. 1998*c. J. Clin. Invest.* (*in press*)
74. Manavalan, P., Dearborn, D.G., McPherson, J.M., Smith, A.E. 1995. *FEBS Lett.* **366:**87–91
75. McNicholas, C.M., Guggino, W.B., Schwiebert, E.M., Hebert, S.C., Giebisch, G., Egan, M.E. 1996. *Proc. Natl. Acad. Sci. USA* **93:**8083–8088
76. McNicholas, C.M., Nason, M.W., Jr., Guggino, W.B., Schwiebert, E.M., Hebert, S.C., Giebisch, G., Egan, M.E. 1997. *Am. J. Physiol.* **273:**F843–8
77. Merlin, D., Jiang, L., Strohmeier, G.R., Nusrat, A., Alper, S.L., Lencer, W.I., Madara, J.L. 1998. *Am. J. Physiol.* **275:**C484–95
78. Moyer, B.D., Loffing, J., Schwiebert, E.M., Loffing-Cueni, D., Halpin, P.A., Karlson, K.H., Ismailov, I.I., Guggino, W.B., Langford, G.M., Stanton, B.A. 1998. *J. Biol. Chem.* **273:**21759–21768
79. Muimo, R., Banner, S.J., Marshall, L.J., Mehta, A. 1998. *Am. J. Respir. Cell. Mol. Biol.* **18:**270–278
80. Naren, A.P., Nelson, D.J., Xie, W., Jovov, B., Pevsner, J., Bennett, M.K., Benos, D.J., Quick, M.W., Kirk, K.L. 1997. *Nature* **390:**302–305
81. Novak, I., Greger, R. 1988. *Pfluegers Arch.* **411:**546–553
82. Okamoto, T., Murayama, Y., Hayashi, Y., Inagaki, M., Ogata, E., Nishimoto, I. 1991. *Cell* **67:**723–730
83. Orlando, R.C., Powell, D.W., Croom, R.D., Berschneider, H.M., Boucher, R.C., Knowles, M.R. 1989. *Gastroenterology* **96:**1041–1048
84. Palmer, L.G., Sackin, H., Frindt, G. 1998. *J. Physiol.* **509:**151–162
85. Pier, G.B., Grout, M., Zaidi, T.S. 1997. *Proc. Natl. Acad. Sci. USA* **94:**12088–12093
86. Poulsen, J.H., Fischer, H., Illek, B., Machen, T.E. 1994. *Proc. Natl. Acad. Sci. USA* **91:**5340–5344
87. Prat, A.G., Xiao, Y.-F., Ausiello, D.A., Cantiello, H.F. 1995. *Am. J. Physiol.* **268:**C1552–C1561
88. Randak, C., Neth, P., Auerswald, E.A., Eckerskorn, C., Assfalg-Machleidt, I., Machleidt, W. 1997. *FEBS. Lett.* **410:**180–186
89. Reddy, M.M., Quinton, P.M., Haws, C., Wine, J.J., Grygorczyk, R., Tabcharani, J.A., Hanrahan, J.W., Gunderson, K.L., Kopito, R.R. 1996. *Science* **271:**1876–1878
90. Ruknudin, A., Schulze, D.H., Sullivan, S.K., Lederer, W.J., Welling, P.A. 1998. *J. Biol. Chem.* **273:**14165–14171
91. Sanguinetti, M.C., Curran, M.E., Zou, A., Shen, J., Spector, P.S., Atkinson, D.L., Keating, M.T. 1996. *Nature* **384:**80–83
92. Santos, G.F., Reenstra, W.W. 1994. *Biochim. Biophys. Acta* **1195:**96–102
93. Schoumacher, R.A., Shoemaker, R.L., Halm, D.R., Tallant, E.A., Wallace, R.W., Frizzell, R.A. 1987. *Nature* **330:**752–754
94. Schreiber, R., Greger, R., Kunzelmann, K. 1997. *Pfluegers Arch.* **434:**841–847
95. Schreiber, R., Greger, R., Ricken, S., Nitschke, R., Kunzelmann, K. 1998*a. Pfluegers Arch.* **435:S1:**P11-2(*Abstr.*)
96. Schreiber, R., Nitschke, R., Greger, R., Kunzelmann, K. 1998*b. J. Biol. Chem.* (*in press*)
97. Schwiebert, E.M., Egan, M.E., Hwang, T.-H., Fulmer, S.B., Allen, S.S., Cutting, G.R., Guggino, W.B. 1995. *Cell* **81:**1063–1073
98. Seidler, U., Blumenstein, I., Kretz, A., Viellard-Baron, D., Rossmann, H., Colledge, W.H., Evans, M., Ratcliff, R., Gregor, M. 1997. *J. Physiol.* **505:**411–423
99. Seksek, O., Biwersi, J., Verkman, A.S. 1996. *J. Biol. Chem.* **271:**15542–15548
100. Shimkets, R.A., Lifton, R.P., Canessa, C.M. 1997. *J. Biol. Chem.* **272:**25537–25541
101. Short, D.B., Trotter, K.W., Reczek, D., Kreda, S.M., Bretscher, A., Boucher, R.C., Stutts, M.J., Milgram, S.L. 1998. *J. Biol. Chem.* **273:**19797–19801
102. Smith, J.J., Travis, S.M., Greenberg, E.P., Welsh, M.J. 1996. *Cell* **85:**229–236

103. Spilmont, C., Hinnrasky, J., Zahm, J.M., Jacquot, J., Puchelle, E., Kuhry, J.G. 1996. *Biochem. Biophys. Res. Commun.* **227:**182–188
104. Stutts, M.J., Canessa, C.M., Olsen, J.C., Hamrick, M., Cohn, J.A., Rossier, B.C., Boucher, R.C. 1995. *Science* **269:**847–850
105. Stutts, M.J., Rossier, B.C., Boucher, R.C. 1997. *J. Biol. Chem.* **272:**14037–14040
106. Takahasi, A., Watkins, S.C., Howard, M., Frizzell, R.A. 1996. *Am. J. Physiol.* **271:**C1887–C1894
107. Todd-Turla, K.M., Rusvai, E., Naray-Fejes-Toth, A., Fejes-Toth, G. 1996. *Am. J. Physiol.* **270:**F237–44
108. Treharne, K.J., Marshall, L.J., Metha, A. 1994. *Am. J. Physiol.* **267:**L592–601
109. Valverde, M.A., Diaz, M., Sepulveda, F.V., Gill, D.R., Hyde, S.C., Higgins, C.F. 1992. *Nature* **355:**830–833
110. Valverde, M.A., O'Briens, J.A., Sepulveda, F.V., Ratcliff, R.A., Evans, M.J., Colledge, W.H. 1995. *Proc. Natl. Acad. Sci.* **92:**9038–9041
111. Wang, S., Raab, R.W., Schatz, P.J., Guggino, W.B., Li, M. 1998. *FEBS. Lett.* **427:**103–108
112. Warth, R., Greger, R. 1993. *Cell Physiol Biochem.* **3:**2–16
113. Warth, R., Riedemann, N., Bleich, M., Van Driessche, W., Busch, A.E., Greger, R. 1996. *Pfluegers Arch.* **432:**81–88
114. Welsh, M.J. 1986. *Science* **232:**1648–1649
115. Willumsen, N.J., Boucher, R.C. 1992. *J. Physiol.* **455:**247–269
116. Wilschanski, M.A., Rozmahel, R., Beharry, S., Kent, G., Li, C., Tsui, L.-C., Durie, P., Bear, C.E. 1996. *Biochem. Biophys. Res. Com.* **219:**753–759
117. Winpenny, J.P., Verdon, B., McAlroy, H.L., Colledge, W.H., Ratcliff, R., Evans, M.J., Gray, M.A., Argent, B.E. 1995. *Pfluegers Arch.* **430:**26–33
118. Yun, C.H., Oh, S., Zizak, M., Steplock, D., Tsao, S., Tse, C.M., Weinman, E.J., Donowitz, M. 1997. *Proc. Natl. Acad. Sci. USA* **94:**3010–3015
119. Zdebik, A., Hug, M.J., Greger, R. 1997. *Pfluegers Arch.* **434:**188–194
120. Zhang, Y., Yankaskas, J.R., Wilson, J., Engelhardt, J.F. 1996. *Am. J. Physiol.* **270:**C1326–C1335