CFTR: A chloride channel, channel regulator, or both?

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In this session, new insights in the structure, the function and the regulation of cystic fibrosis transmembrane conductance regulator (CFTR) were discussed. A special emphasis was to examine in more detail the recent findings on the interaction between CFTR and the epithelial sodium channel (ENaC). How does ENaC interact with CFTR? Is this interaction direct or indirect? What is the physiological and pathophysiological relevance of the ENaC/CFTR interaction in different epithelial tissues?

John R. Riordan (Scottsdale, Arizona, USA) reported that CFTR has several features that are novel among ABC proteins and among ion channels. Indeed, CFTR is the only known ABC protein to form an ion conduction pore and one of two known channel-forming polypeptides to have enzymatic activity. To address the possible relationship between these two activities of the protein, Riordan and colleagues have analyzed nucleotide binding, hydrolysis and dissociation, as well as channel gating by native membrane-bound CFTR and hydrolysis by the purified protein after reconstitution in lipid membranes. The data indicate that the two activities are indeed related. However, hydrolysis is not essential to either the initiation or termination of gating; binding and dissociation are sufficient and hydrolysis accelerates the latter. Thus, CFTR has adapted the ABC protein structural architecture to form a ligand gated ion channel in which hydrolysis of the ligand facilitates ligand dissociation and provides reversibility of the gating cycle [1, 2]. Riordan and colleagues have also employed both biochemical and functional analyses to evaluate CFTR quaternary structure. As yet, there is no compelling evidence that a homo-oligomeric structure is required for CFTR function.

Malla Reddy Madireddi (San Diego, California, USA) is studying the role of CFTR in transepithelial sodium absorption in the ducts of sweat glands. Early studies by Boucher and colleagues [3] suggested that a significant cross talk occurs between CFTR and ENaC. It was shown that activating CFTR inhibits ENaC channel so that activation of CFTR and deactivation of ENaC is reciprocal. This scheme was proposed to explain the disturbance in airway surface liquid in CFTR. Reddy and colleagues have determined if these events would hold true in a purely absorptive epithelium, namely the native human sweat gland ducts. They found that, in contrast to reciprocal activities, activating CFTR by either cAMP, cGMP or the G-proteins plus 5 mmol/L ATP was accompanied by concomitant activation and not inhibition of ENaC conductance [4]. These results from a purely absorptive epithelium raise the question of whether simultaneous activation (as opposed to reciprocal inhibition) of CFTR and ENaC is a generalized property of other epithelial cells with similar absorptive function.

Richard Boucher (Chapel Hill, North Carolina, USA) discussed the two main hypotheses that have been proposed to explain the role of CFTR in air surface liquid (ASL) physiology and lung defense. The “compositional” hypothesis predicts that ASL is kept low (<50 mmol/L) to permit antimicrobial factors to act as a chemical shield. This hypothesis predicts active transcellular Na⁺ absorption with passive transcellular Cl⁻ movement via the CFTR Cl⁻ channel. Water permeability is maintained on airway surfaces not by the impermeability of the airway epithelium, as in the sweat duct, but due to putative passive “surface forces.” The second hypothesis (volume hypothesis) predicts that ASL volume (height) is regulated by active ion transport to maintain efficient mechanical mucus clearance as a primary form of lung defense. In this scenario, CFTR has dual functions: (1) a regulator of the epithelial Na⁺ channel activity; and (2) a Cl⁻ channel. In this scenario, the net movement of water is predicted to follow salt transport and hence, isotonic solutions are predicted on normal airway surfaces. Boucher described recent evidence from his lab supporting the second hypothesis [5]. In cultured airway epithelia that had maintained a high degree of differentiation, Boucher and colleagues showed that liquid is absorbed until the ASL volume equals the height of the extended cilium, that is, they maintain a 7-µ deep periciliary liquid (PCL) layer [6]. Volume absorption is mediated via transepithelial Na⁺ transport, with the apical membrane ENaC, the rate-limiting element. Interestingly, as ASL volume/height approaches that of PCL,
ENaC activity is inhibited, slowing the rate of Na\(^+\) absorption and generating driving forces for the initiation of CFTR-mediated Cl\(^-\) secretion. In contrast to normals, CF airway epithelia absorb more rapidly, reflecting the absence of CFTR inhibition of ENaC, and fail to initiate Cl\(^-\) secretion, reflecting the absence of CFTR Cl\(^-\) channel function. The combined effects of raised Na\(^+\) transport and failure to secrete Cl\(^-\) deplete PCL, which is the initiating CF lesion that leads to the failure of mucus clearance and chronic infection.

Karl Kunzelmann (St. Lucia, Australia) studied the inhibition of ENaC by CFTR in the Xenopus oocyte expression system. The group demonstrated that ENaC is inhibited during stimulation of CFTR, independent of the experimental setup and the magnitude of the whole cell current at variance with a previous report [7]. However, a minimal Cl\(^-\) current is required for the inhibition of ENaC, which is augmented at higher CFTR to ENaC current ratios [8]. Similar to CFTR, another Cl\(^-\) channel (CIC-0) inhibits ENaC, as well as high extracellular Na\(^+\) and Cl\(^-\) in partially permeabilized oocytes. The authors conclude that inhibition of ENaC is not specific to CFTR and seems to be mediated by Cl\(^-\) entry, reminiscent of the Cl\(^-\) feedback regulation observed in mouse mandibular ducts. The data obtained so far are consistent with a charge interaction of Cl\(^-\) ions with the epithelial sodium channel. The nature of this interaction remains elusive.

Christoph Korbmacher (Oxford, England, United Kingdom) confirmed previous reports that in oocytes co-expressing ENaC and CFTR, the amiloride-sensitive current was reduced during cAMP mediated stimulation of CFTR. Using a novel chemiluminescence assay [9], the group demonstrated that this CFTR-dependent inhibition of ENaC currents was not associated with a decrease in ENaC surface expression, suggesting that the inhibitory effect is due to a decrease in channel open probability. Korbmacher showed that the secretory K\(^+\) channel (Kir1.1/ROMK) was also regulated by ENaC. It was concluded that ENaC up-regulates ROMK1 in a CFTR dependent manner. CFTR, therefore, may provide the mechanistic link that mediates the coordinated up-regulation of ROMK during the stimulation of ENaC by hormones such as aldosterone or vasopressin.

Finally, Jean-Daniel Horisberger (Lausanne, Switzerland) built a mathematical model of an epithelial cell monolayer comprising variable apical Cl\(^-\) and Na\(^+\) conductances, a basolateral Na,K-pump, basolateral Cl\(^-\) and K\(^+\) conductances and a shunt conductance. This model permits stimulation of the ion transport under current or voltage clamp conditions and can monitor individual ionic membrane currents, membrane potentials and intracellular ion concentrations. The model shows that, with regards to ENaC and CFTR expressed in the same apical membrane, electrical coupling of these two ion channels can explain a large part of the effects of CFTR activation on the Na\(^+\) current flowing through ENaC.

In summary, the most relevant questions in this field were discussed extensively and despite the fact that no final answers were offered, a better picture of what should be done in the future was provided. The discussion pointed to the critical importance of a clear understanding of where and how CFTR and ENaC function. Everybody agreed that a precise localization and ultimately co-localization of ENaC and CFTR in the apical versus basolateral membrane of various epithelia is surely a prerequisite to improve our understanding in this field. Unfortunately, the lack of immunological probes (antibodies against CFTR and/or against ENaC) thus far has prevented data of sufficient quality to be obtained so that a precise cellular and subcellular expression of the two proteins in a given native epithelial cell can be established. It is expected that CFTR/ENaC interactions will be tissue and cell specific. To understand CF pathophysiology, two major key areas have to be elucidated: (1) the “sensors” that are able to sense ASL volume and transmit this information to the epithelial effectors, that is, ENaC and CFTR, and (2) the mechanisms by which CFTR inhibits ENaC function (directly by protein-protein interaction or indirectly by an intracellular signaling cascade). These are nice challenges for future research.

REFERENCES

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