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VIP as a Corrector of CFTR Trafficking and Membrane Stability

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1. Introduction

Cystic Fibrosis (CF) is a fatal autosomal recessive disease characterized by abnormal ion transport across epithelia, viscous mucus secretions, chronic bacterial infections, and inflammation in the airways that result from misprocessed or nonfunctional CFTR (Cystic Fibrosis Transmembrane conductance Regulator) chloride channels, normally located at the apical membrane of epithelial cells in exocrine tissues. CFTR activity is regulated by the Vasoactive Intestinal Peptide (VIP), a neuropeptide with potent anti-inflammatory, bronchodilatory and immunomodulatory functions. In airway sub-mucosal glands and other exocrine tissues, VIP is the major physiological activator for CFTR-dependent secretions, which contribute to local innate defense. When CFTR is defective or absent from the apical membrane of epithelial cells, due to mutations in the CFTR gene, airway glands no longer secrete in response to VIP stimulation and synergy with acetylcholine is lost. Although it is thought that VIP receptors are not altered in CF epithelial tissues, early studies have demonstrated that innervations by VIP-containing nerve fibers of the skin sweat glands, nasal and intestinal mucosa of CF patients is almost absent compared to healthy individuals, suggesting that absence of VIP stimulation could play a central role in the development of CF pathology.

Our group has recently demonstrated that VIP regulates CFTR membrane stability via activation of the $VPAC_1$ receptor and the $G_{\alpha i/q}$ signaling cascade in a PKCε-dependent manner. We also found that prolonged VIP exposure can rescue trafficking to the cell membrane and function of ΔF508-CFTR channels; the most commonly found mutation in CF. Our most recent *in vivo* studies using VIP knock-out (KO) mice provides clear evidence of the importance of VIP in maintaining healthy exocrine tissues, and the molecular link, between the absence of VIP stimulation and the development of a CF-like phenotype. We also observed a corrective effect with exogenous VIP administration, which restored normal trafficking and stabilized functional CFTR channels at the apical membrane of epithelial cells of the lung and small intestine (Fig. 1&2).

This mini-review summarizes recent and past findings on the role VIP in CFTR regulation and how it relates to the development of CF.

2. VIP historical background: Discovery as a vasodilator peptide in lung and intestine and rediscovery as a neuropeptide

First discovered as a smooth-muscle-relaxant, vasodilator peptide in the lung (Said, 1969), VIP was soon thereafter isolated from porcine intestine (Said and Mutt, 1970), chemically characterized (Mutt and Said, 1974), and synthesized (Bodanszky *et al*, 1973).

A 28 amino-acid residue peptide, VIP is structurally related to several other peptides, said to make up a "family," including pituitary adenylate cyclase-activating peptides (PACAP) 27 & 38, secretin, glucagon, helodermin, sauvagine, urotensin I, and gastric inhibitory peptide (glucose-dependent insulinotropic peptide) (Said, 2006).

With the aid of specific radioimmunoassay and immunofluorescence techniques, VIP immunoreactivity was detected in normal tissues and organs outside of the gastrointestinal tract, and found at high concentrations, as well as in certain neurogenic and endocrine tumors associated with excessive VIP secretion and high plasma levels (Said and Faloona, 1975). Eventually, the peptide was "rediscovered" in normal brain and peripheral nerves (Said and Rosenberg, 1976), and its true identity was recognized as a neuropeptide with neurotransmitter or neuromodulator properties. VIP is now considered to have physiologic regulatory influences on multiple organ systems, to be involved in the pathogenesis of several human disorders, and to have potential therapeutic benefit in a variety of disorders (Said, 1991b).

3. Role of VIP in exocrine secretion

As a neuropeptide, VIP was found to richly innervate all exocrine glands, including the pancreas, sweat, salivary, lachrymal, bronchial, and intestinal glands. Investigators learned that VIP worked in unison with cholinergic nerves, serving primarily to promote blood flow, and together with acetylcholine, to regulate and coordinate exocrine function (Lundberg *et al*, 1980). Evidence was accumulating that VIP, acting via receptors on these glands (Heinz-Erian *et al*, 1986), stimulated water and chloride transport across intestinal and tracheobronchial mucosa, $HCO3^-$ secretion by pancreatic acini, and promoted the movement of water and chloride across other epithelial surfaces (Heinz-Erian *et al*, 1985).

4. Is there a link to CF?

The above observations suggested that VIP exerted a regulatory influence on exocrine function, that appeared to run opposite to the observed defects in CF. With my associates (Said & colleagues), therefore, we postulated that the exocrine abnormalities of CF might be caused by a deficiency of VIP innervation. Accordingly, we examined the presence, distribution, and density of VIP-immunoreactive nerves supplying the sweat glands of normal subjects and CF patients. We selected sweat glands because: a) they express one of the cardinal functional abnormalities of the disease; b) unlike other exocrine organs involved in the disease, such as the lungs, sweat glands remain free of infection or morphologic changes; and c) they are easily accessible through skin biopsy (Heinz-Erian *et al*, 1985).

4.1 Deficient VIP – Containing nerves in CF exocrine tissues

Normal skin showed a rich network of VIP-immunoreactive nerves around secretory sweat gland acini, and a moderate innervation of the reabsorptive ducts. Individual VIP-positive nerve fibers were closely associated with basement membrane of both acini and duct cells. VIP innervation in CF samples, by contrast, was either absent or minimal both in the acini and in the ducts (Heinz-Erian *et al*, 1985).

Other than the skin, VIPergic neurons are also present in other sites of important CF manifestation such as the mucosa of the small intestinal, the pancreas and the respiratory epithelium. As observed in the skin, a deficiency, specifically in VIP-immunoreactive nerves, was observed in the nasal and intestinal mucosa of CF patients (Wattchow *et al*, 1988) while other types of nerve fibers were still present. The loss of VIP-immunoreactive nerve fibers was not however generalized and normal innervation was observed in intestinal muscles.

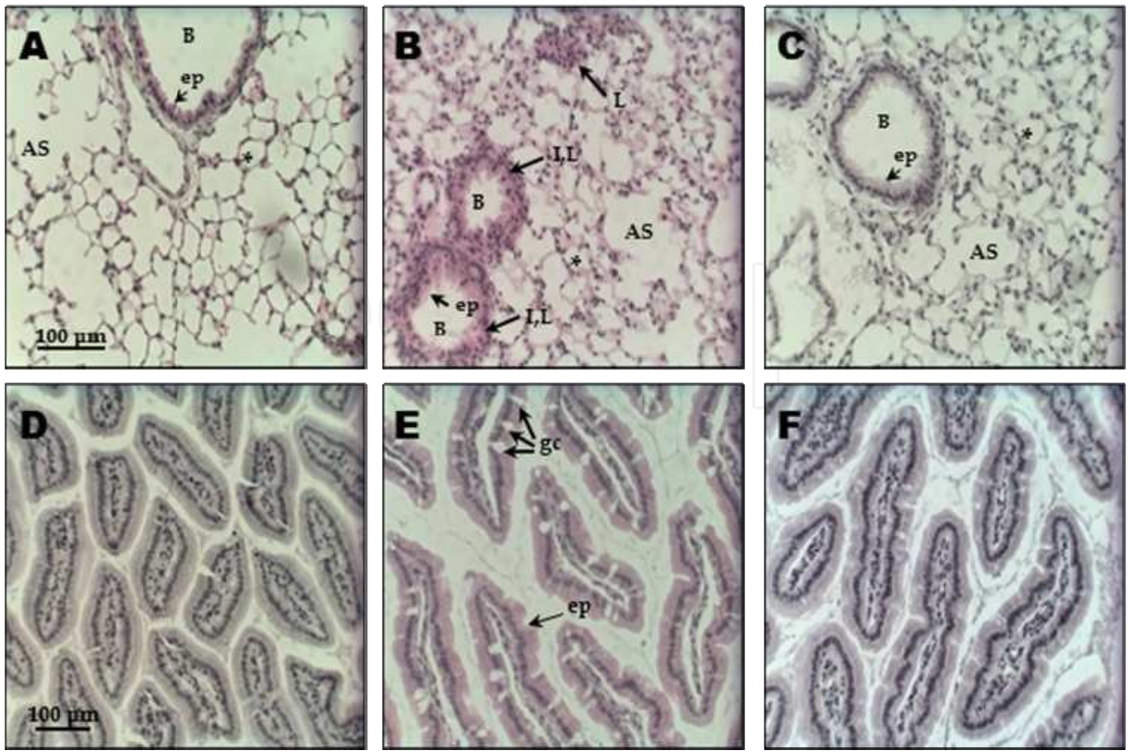
These findings, later confirmed by other investigators (Savage *et al*, 1990), raised the questions: 1) Is the decreased VIP innervation of CF glands and ducts causally related to the chloride ion abnormality in CF? and 2) what is the role of VIP, if any, in the pathogenesis, or correction, of CF pathology? Both questions remained unanswered for many years to follow.

4.2 Changes in CF submucosal glands

In response to VIP stimulation, normal human submucosal glands, in which CFTR is highly expressed, secrete low level of mucus which participates in the airways innate defense noteworthy by enabling mucociliary clearance (Wine, 2007). In CF glands, however, mucous secretion in response to VIP or cAMP elevation is altered (Joo *et al*, 2002). Mucus becomes more acidic (Song *et al*, 2006) and more viscous (Jayaraman *et al*, 2001) compared to normal. The same dysfunction is observed in CFTR-KO mice (Ianowski *et al*, 2007). VIP and cholinergic agonists synergistically induce mucous secretion from healthy human and pig glands but this synergy is absent in CF tissues (Choi *et al*, 2007). However, the secretion of large amounts of mucus in response to acute stimulation, primarily under the vagal pathway[1], and cholinergic stimulation are still present although altered (Wine, 2007).

5. Recent confirmation of a link, from studies of VIP and VIP-KO mice

We have recently used VIP knockout C57/Bl6 mice to demonstrate *in vivo* the central role of VIP in CFTR regulation and exocrine epithelial tissue integrity. These mice have been proven to be a very good model for airways diseases such as bronchial asthma (Szema *et al*, 2006; Hamidi *et al*, 2006; Said, 2009). They display airways inflammation and hyper-responsiveness to methacholine. They also present moderate pulmonary hypertension, right ventricular hypertrophy, and thickened pulmonary arteries (Said et al, 2007). We have used H&E staining for pathological assessment of the lung, small intestine and pancreas (Fig. 1). Interestingly, changes observed resembled those seen in CF. VIP-KO intestinal tissues had a significant increase in goblet and inflammatory cells. In the lung, we observed lymphocyte aggregation, increased airway secretion, alveolar thickening and edema. The pancreas presented increased secretion and increased infiltration with inflammatory cells surrounding ducts. These pathological changes could be reversed, closed to a wild-type phenotype, by VIP treatment consisting of intra-peritoneal injections of VIP (15μg) every other day for 3 weeks (Fig. 1) (Alcolado, 2010).

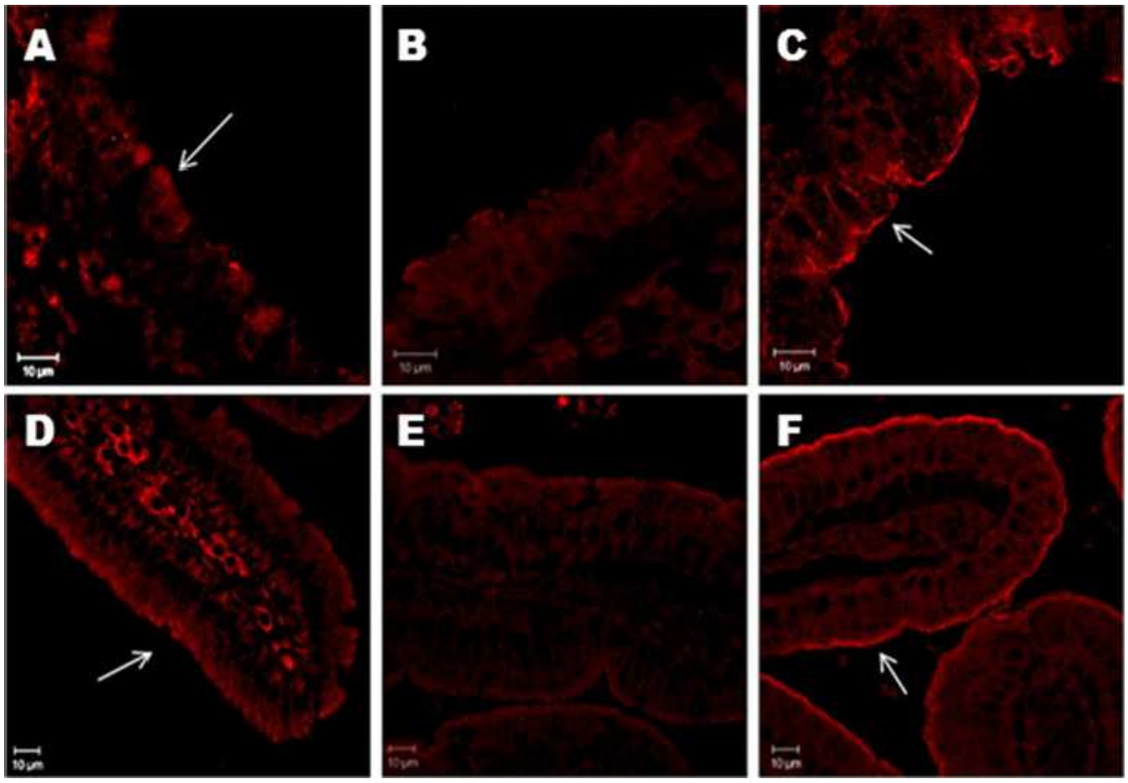


Histology sections of lung (upper panels) and duodenum (lower panels) from wild-type (**A** & **D**), VIP-KO (**B** & **E**) and VIP-KO treated (**C** & **F**) mice. Samples were embedded in paraffin before sectioning. 5 µm thick sections were mounted onto microscopy slides before hematoxylin and eosin (H & E) staining. Images were taken with a light microscope at 20X magnification. Compared to normal tissue, VIP-KO lungs show signs of inflammation (I) and lymphocyte aggregation (dark blue staining), thickening of the mucosa around bronchiolar space, thickening of the alveolar walls and the presence of inflammatory cells. The bronchiolar epithelium (ep) is also damaged. All these pathological signs are reversed to normal after VIP treatment (**C**). In the duodenum, transversal sections show increased amount of goblet cells and epithelium damage in the upper villi of VIP-KO mice tissues. As for the lung, these pathological signs are reversed by VIP treatment.
AS= alveolar sac, B = bronchioles, ep=epithelium, gc = goblet cells, I = inflammation, L = lymphocytes aggregation, * alveoli.

Fig. 1. VIP-KO mice lung and duodenum present pathological signs reversible by VIP treatment (adapted from Alcolado, 2010).

VIP binds to class II seven transmembrane spanning domain G protein-coupled receptors (GPCR) on the basolateral membrane of epithelial cells (Laburthe *et al*, 2007, 2002; Dickson *et al*, 2006; Chastre *et al*, 1989). VIP can bind to 3 receptors: $VPAC_1$, $VPAC_2$ and PAC_1. The highest affinity is for $VPAC_1$ (EC_{50} < 0.1nM) followed by $VPAC_2$ (EC_{50} =10 nM) and very little affinity for PAC_1 (EC_{50} ~40 nM). The PAC_1 receptor has a much greater affinity for Pituitary Adenylate Cyclase-Activating Polypeptide (EC_{50} ~0.2 nM) (Dickson and Finlayson, 2009). Other members of this peptide family, such as secretin and helodermin, can also bind to VPAC receptors although with much lower affinity than VIP (Laburthe *et al*, 2007) . Although VIP innervating fibres were found to be absent from CF intestinal mucosa (Wattchow *et al*, 1988), a study on CF foetuses revealed the presence of VIP receptors with unaltered pharmacology in the small intestine (Chastre *et al*, 1989).

In the VIP-KO mice model, we confirmed the expression of $VPAC_1$, $VPAC_2$ and PAC_1 by RT-PCR. Immunoblotting of lung and duodenum tissue lysates revealed unchanged PAC_1 expression in VIP-KO mice tissues compared to wild-type (WT), whereas VPAC 1 and 2 were found to be more abundant in VIP-KO tissues. These 2 receptors expression level remained up-regulated after VIP treatment (Conrad, 2011). CFTR localization was examined by immunostaining followed by confocal microscopy (Fig. 2). WT tissues showed CFTR predominantly at the apical membrane of epithelial cells in contrast to VIP-KO tissues, where CFTR distribution was mainly observed intracellularly. No changes in CFTR protein abundance or maturation were observed in immunoblots. Interestingly, VIP treatment restored strong CFTR membrane localization (Fig. 2), confirming the important role of VIP chronic exposure to maintain CFTR channels at the membrane, where it can exert its function, and for exocrine epithelial tissues integrity. Inflammation and damage observed in VIP-KO tissues can be attributed, at least in part, to the lack of CFTR-dependent secretions which ultimately depend on VIP stimulation both for acute and long-term regulation of CFTR function. These observations provide evidence of the molecular link between early observations of deficient VIP-containing fibers innervation of epithelial layers of exocrine organs in CF tissues and the absence of CFTR-dependent secretions.



Paraffin embedded tissues (lung: upper panels, duodenum: lower panels) were sliced into 5 µm sections and mounted onto microscopy slides before immunostaining with the monoclonal anti-CFTR antibody MAB1660 (1:100). Arrows indicate apical CFTR signal. A & D: wild-type, B & E: VIP-KO, C & F: VIP-KO treated mice.

Fig. 2. CFTR localization at the apical membrane of epithelial cells is lost in VIP-KO tissues and restored by VIP treatment (adapted from Alcolado, 2010).

6. Molecular role of VIP in CFTR regulation

6.1 Regulation of CFTR activation by VIP acute stimulation

CFTR is activated mainly by protein kinase A (PKA)-dependent phosphorylation, with protein kinase C (PKC) stimulation playing an enhancing and permissive role to subsequent responsiveness to PKA (Chappe *et al*, 2008; Jia *et al*, 1997), in part through direct phosphorylation of conserved consensus sequences in CFTR Regulatory (R) domain (Chappe *et al*, 2003; Chappe *et al*, 2004). Hormones and neurotransmitters, such as VIP, which raise cellular cyclic AMP level, can stimulate acute CFTR channel activity. VIP is the most abundant peptide in the airways and the $VPAC_1$ receptor has been shown to stimulate CFTR-dependent chloride secretion upon VIP binding through activation of both PKA- and PKC-dependent signaling pathways in airway submucosal glands epithelial cell line Calu-3 (Chappe *et al*, 2008; Derand *et al*, 2004). Although class II GPCR are generally coupled to $G_{\alpha s}$[2] and adenylate cyclase activation to increase intracellular cAMP content, numerous reports have demonstrated that VIP receptors can couple to alternate G proteins and elicit signaling cascades cross-talk involving $G_{\alpha i/q}$[2], PKC and calcium release on top of the conventional $G_{\alpha s}$ and cAMP cascade (Derand *et al*, 2004; Bewley *et al*, 2006; Chappe *et al*, 2008; Sreedharan *et al*, 1994; Xia *et al*, 1996; Shreeve *et al*, 2000; Rafferty *et al*, 2009) (Fig. 3).

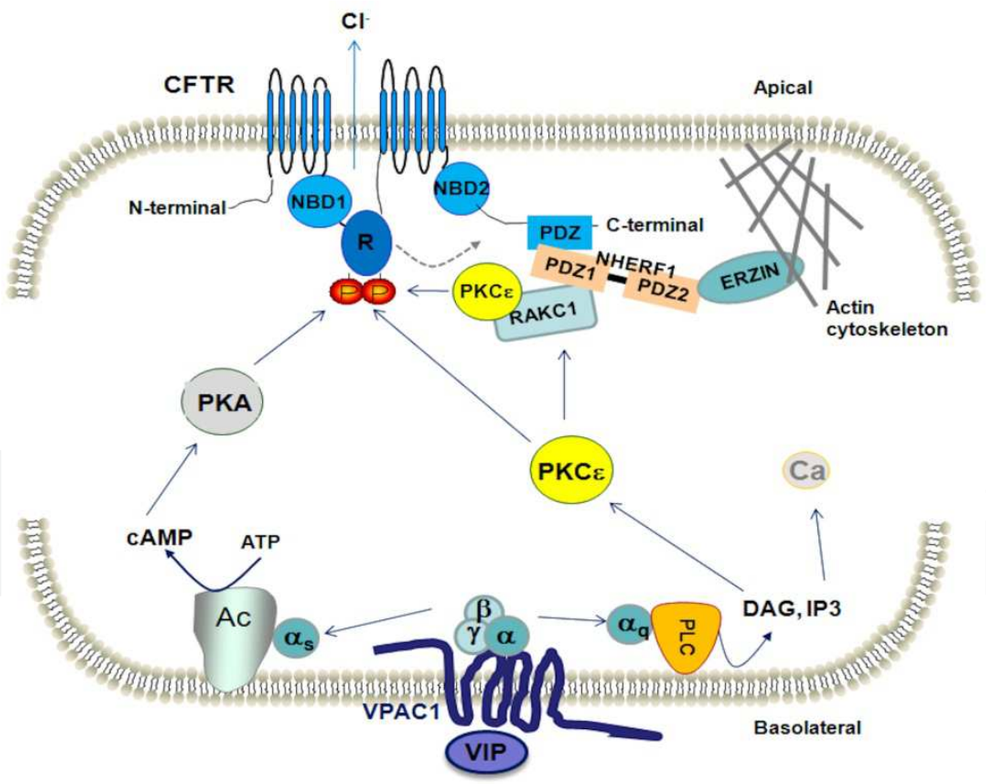


Fig. 3. CFTR regulation by VIP.

After binding to the $VPAC_1$ receptor, on the basolateral membrane, VIP induces the activation of both PKA and PKC signalling cascades. On the right end side of this cartoon is displayed the PKCε cascade of activation, as observed in our experiments and by others. The $G\alpha_q$ protein

activates phospholipase C (PLC) which produces inositol tri-phosphate (IP3) and diacylglycerol (DAG). IP3 induces calcium release whereas DAG stimulates PKCε. PKCε can directly phosphorylate membrane CFTR on specific sites in the Regulatory domain. The activated PKCε also binds to the receptor for active kinase C (RAKC1) and translocate to the plasma membrane where RAKC1 binds to the PDZ domain 1 of NHERF1 (Na+/H+ Exchanger Regulatory Factor-1). This complex interacts with CFTR C-terminal, on a PDZ motif, and to ERZIN which anchors the whole complex to the actin cytoskeleton. This regulation maintains CFTR at the membrane and reduces its endocytosis. The co-localization of PKCε and CFTR is thought to maintain CFTR phosphorylation which contributes to its activation. We observed that the phosphorylated R domain relocates from its initial position, close to the Nucleotide Binding domains (NBD), to a new position with increased binding strength. We hypothesize here that re-binding of the phosphorylated R domain will further increase CFTR stability at the membrane through interaction with the PKCε-RAKC1-NHERF1 complex. In parallel (see left side), the $VPAC_1$ receptor can activate the PKA signalling cascade by associating with the $G\alpha_s$ protein which activates Adenylyl cyclase (Ac) to produce intracellular cAMP. This second messenger stimulates PKA. Direct phosphorylation of the CFTR R domain by PKA activates the channel gating. CFTR gating is further enhanced by PKC phosphorylation.

6.2 Role of VIP in CFTR membrane insertion and stability

Control of CFTR recycling is an important mechanism for the regulation of CFTR-dependent secretions. Adapters and proteins involved in CFTR endocytosis have been studied in detail (Ameen et al, 2007; Okiyoneda and Lukacs, 2007), but its regulation by physiological agonists is far less well understood and seems to be cell-type specific. Although CFTR function as a chloride channel requires apical membrane localization to participate in the regulation of exocrine secretions, it is mostly present in recycling endosomes, forming an important submembranar pool of mature proteins (Bradbury *et al*, 1994; Bradbury and Bridges, 1994; Webster *et al*, 1994). CFTR has a relatively long half-life: 16-24 hrs and a rapid turnover, although variable among cell lines: 2-16% per minute (Chappe *et al*, 2008; Lukacs *et al*, 1993; Swiatecka-Urban *et al*, 2005). CFTR is internalized by clathrin coated vesicles due to the presence of a dileucine and tyrosine endocytotic signals in its C-terminal. The tyrosine based motif interacts with the clathrin adapter complex AP-2 to enter into clathrin coated pits. CFTR also interacts with actin-binding proteins like myosin VI and to N-SWAP to recycle back to the plasma membrane via recycling endosomes (Okiyoneda and Lukacs, 2007; Swiatecka-Urban *et al*, 2004; Ganeshan *et al*, 2006). Conflicting results exist regarding the role of cAMP or other second messengers in CFTR recycling probably due to disparity in epithelial cells studied or the use of over-expressing systems which might saturate the normal pathway for CFTR trafficking. Both PKA and PKC-dependent mechanisms have been reported.

6.2.1 In intact tissues

Spiny dogfish shark (*Squalus acanthias)* rectal glands highly regulate salt secretion upon hormonal signals. These glands express a CFTR ortholog with 72% identity to the human CFTR. Acute VIP stimulation of these glands produces an increase in CFTR-mediated chloride secretions. Immunofluorescence labeling also revealed a redistribution of CFTR from intracellular to apical membrane localization following VIP stimulation (Lehrich *et al*, 1998).

In rats, a subpopulation of epithelial cells found in the small intestinal villi was identified as CFTR High Expresser cells (CHE) (Ameen *et al*, 1995). In response to VIP stimulation, CFTR present in sub-apical vesicular pool redistributed to the apical membrane but returned to the intracellular pool after removal of VIP (Ameen *et al*, 1999; Ameen *et al*, 2000).

6.2.2 In cell lines

Our lab has established that prolonged VIP stimulation of polarized airway epithelial cells stabilizes CFTR at the cell surface by reducing its internalization rate by more than 50%. The consequence of this regulation is an increase in CFTR-mediated chloride secretion. This was demonstrated initially in Calu-3 cells, a widely used model for submucosal gland serous cells, which express $VPAC_1$ receptors on their basolateral membrane and wild-type CFTR at the apical surface. Analysis of surface proteins by biotinylation and streptavidin extraction methods, revealed a large increase in apical CFTR after VIP exposure which was significant after 10 min and maximal within 2 hrs of VIP treatment. No changes in total CFTR or the proportion of fully glycosylated CFTR were measured in any tested condition, confirming that the VIP regulation was on mature CFTR recycling and did not affect its trafficking. Interestingly, the signaling cascade involved in this mechanism was $VPAC_1$ and $G_{\alpha i}$ mediated and involved the activation of PKC (Chappe *et al*, 2008). Direct activation of PKC by phorbol esters could mimic VIP effect with more than 2 fold increase in apical CFTR after 2 hrs of treatment. Functional evidence of increased membrane CFTR density after PKC stimulation were also reported in the human colon cell line HT29 together with increased mucus secretion (Bajnath *et al*, 1995). Contrary to previous observations in intestinal epithelial cells (Ameen *et al*, 2003; Bradbury and Bridges, 1992), raising intracellular cAMP by forskolin had no effect on the amount of CFTR at the apical membrane of Calu-3 cells. It is thus evident that VIP effect on CFTR membrane insertion is coupled to different signaling pathways in airways and intestinal cells, with the latter having more complex regulation possibly depending on the cellular model considered.

6.2.3 Rescue of ΔF508-CFTR maturation and membrane stability

The most common mutation in CF is the deletion of a phenylalanine residue at position 508 (ΔF508) that causes improper folding of the CFTR protein, resulting in its retention in the endoplasmic reticulum and proteosomal degradation of the majority of the newly synthesized CFTR proteins (Cheng *et al*, 1990; Kartner *et al*, 1992; Penque *et al*, 2000). However, part of the ΔF508-CFTR protein can still mature and reach the cell membrane where it retains some chloride channel function (Bronsveld *et al*, 2000; Penque *et al*, 2000; Kopito, 1999). Many efforts on CF research are devoted to attempt to rescue ΔF508-CFTR defective trafficking to restore normal epithelial function. Interestingly, ΔF508-CFTR retains some chloride channel activity when rescued from degradation by low temperature, chemical chaperones or other correctors. However, the half-life of the mutant protein is considerably shorter than that of the wild-type CFTR, mainly due to instability at the apical membrane (Lukacs *et al*, 1993; Denning *et al*, 1992; Dormer *et al*, 2001; Sharma *et al*, 2001).

We have investigated the potential rescue and stability at the cell membrane of ΔF508-CFTR by VIP treatment in the human nasal epithelial cells JME/CF15, derived from a ΔF508 homozygous patient (Jefferson *et al*, 1990). Immunostaining experiments with specific anti-

CFTR antibodies, followed by confocal microscopy confirmed intracellular localization of ΔF508-CFTR under control conditions, at 37°C, whereas membrane localization was observed in cells cultured at 27°C for 48hrs (Rafferty *et al*, 2009). The important finding of this study was that when JME/CF15 cells, maintained at physiological temperature (37°C), were treated with VIP (300 nM) for 1 or 2 hrs, mature ΔF508-CFTR proteins were observed in western blot experiments and immunostaining confirmed localization at the cell membrane. Functional assays confirmed the presence of CFTR-dependent chloride secretion after VIP treatment at 37°C. In these nasal cells, which express the $VPAC_1$ receptor, we found that VIP-dependent rescue of ΔF508-CFTR trafficking was mediated by the PKA-dependent signaling cascade (Fig. 4). We also found that ΔF508-CFTR membrane insertion obtained at low temperature could be enhanced by prolonged VIP treatment (1 to 2 hrs) which induced a large increase in ΔF508-CFTR function. As previously observed for wild-type CFTR, this regulation involved the $G_{\alpha q}$ and PKC signaling cascade but not the $G_{\alpha s}$ - PKA cascade (Fig. 4).

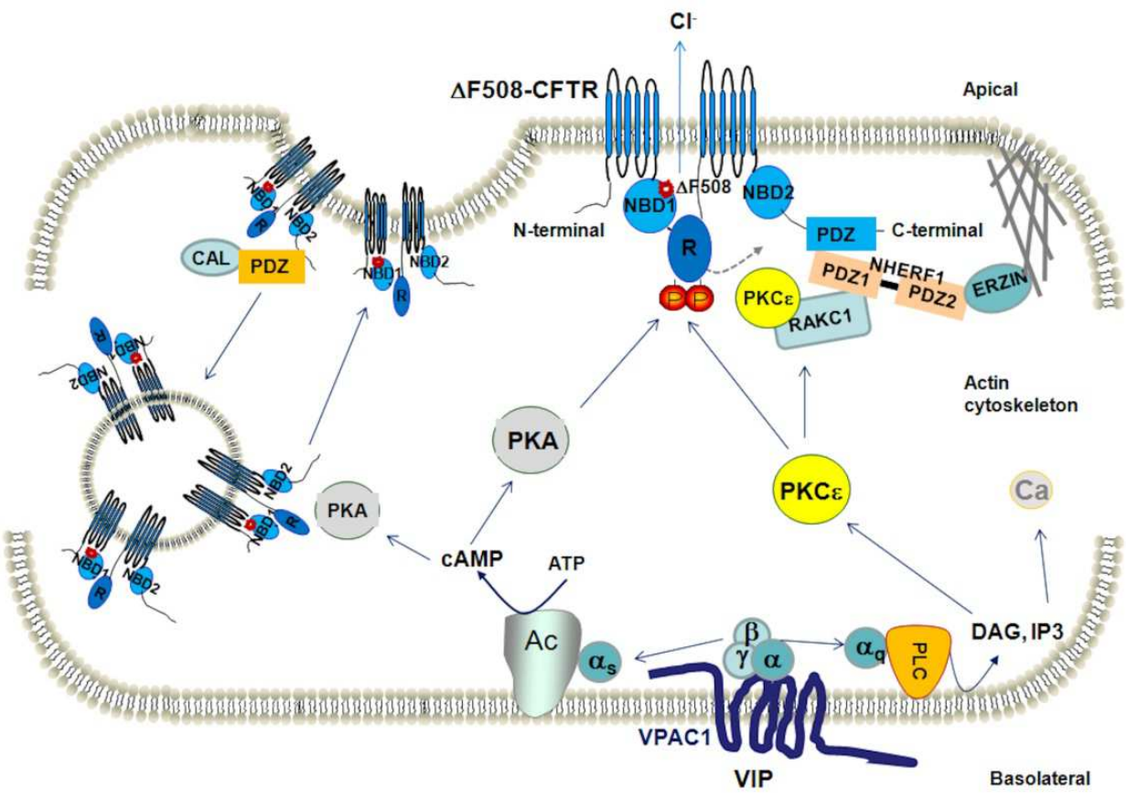


Fig. 4. Regulation of ΔF508-CFTR by VIP.

After binding to the $VPAC_1$ receptor, on the basolateral membrane, VIP induces the activation of both PKA and PKC signalling cascades. On the right side of this cartoon is displayed the PKCε cascade of activation, as observed in our experiments and by others. The $G\alpha_q$ protein activates phospholipase C (PLC) which produces inositol tri-phosphate (IP3) and diacylglycerol (DAG). IP3 induces calcium release whereas DAG stimulates PKCε. PKCε can directly phosphorylate membrane CFTR on specific sites in the Regulatory

domain. The activated PKCε also binds to the receptor for active kinase C (RAKC1) and translocates to the plasma membrane where RAKC1 binds to the PDZ domain 1 of NHERF1. This complex interacts with CFTR C-terminal, on a PDZ motif, and to ERZIN which anchors the whole complex to the actin cytoskeleton. This regulation maintains CFTR at the membrane and reduces its endocytosis. The co-localization of PKCε and CFTR is thought to maintain CFTR phosphorylation which contributes to its activation. We observed that the phosphorylated R domain relocates from its initial position, close to the Nucleotide Binding domains (NBD), to a new unidentified position with increased binding strenght. We hypothesize here that re-binding of the phosphorylated R domain will further increase CFTR stability at the membrane through interaction with the PKCε-RAKC1-NHERF1 complex. In parallel (see left side), the $VPAC_1$ receptor can activate the PKA signalling cascade by associating with the $G\alpha_s$ protein which activates Adenylyl cyclase (Ac) to produce intracellular cAMP. This second messenger stimulates PKA. Direct phosphorylation of the CFTR R domain by PKA activates the channel and is further enhanced by PKC phosphorylation. We also observed that the activated PKA contributes to ΔF508-CFTR trafficking and insertion to the plasma membrane. We hypothesize that PKA effect counteracts the action of the CFTR Associated Ligand (CAL) which had been shown to bind to CFTR C-terminal PDZ motif, and compete with NHERF1, to retain CFTR in endosomes and target ΔF508-CFTR to lysosomal degradation.

Evidence from airway cells thus demonstrates that VIP mechanism of regulation of CFTR activity involves both PKA and PKC signaling in a synergistic manner to rescue defective trafficking of mutant CFTR, activate CFTR gating through direct phosphorylation of its regulatory domain, and most importantly, stabilize CFTR channels at the cell surface by reducing their internalization rate, thus optimizing CFTR-dependent secretions.

6.2.4 Role of PKCε

In both recombinant and native systems, we have investigated the role of PKC on VIP-dependent CFTR membrane stability. PKC isoforms comprise calcium-dependent (α, β_1, β_2, γ) and calcium-independent novel isoforms (δ, ε, η, θ). All necessitate diacyl glycerol to be activated (Reyland, 2009; Dempsey *et al*, 2000; Gallegos and Newton, 2008). With specific inhibitors and siRNA treatments we found that only PKCε, a novel calcium-independent isoform, mediated VIP-dependent increase in CFTR membrane stability in the JME/CF15 epithelial nasal cells and also in the recombinant BHK cells stably expressing wild-type or ΔF508-CFTR (Alcolado *et al*, 2011). This is not surprising as PKCε was previously reported to co-localize with CFTR at the apical membrane of airway epithelial cells and to play a permissive role on CFTR-dependent chloride secretion (Liedtke and Cole, 1998; Liedtke *et al*, 2001; Liedtke *et al*, 2002). The C-terminal of CFTR interacts with either CAL or NHERF1. These two scaffolding proteins regulate CFTR membrane density in an opposite manner. While CAL, which is mostly found in the trans-golgi network, promotes CFTR targeting to lysosomal degradation, NHERF1, which is localized at the cell apical membrane, rather participate in maintaining CFTR at the membrane by tethering it to the actin cytoskeleton (Cheng, J., 2002, 2004). Structural studies indicate that CAL and NEHRF1 might compete for the same binding site in CFTR C-terminal, and their differential interaction with CFTR is thought to regulate the steady-state level of mature CFTR present at the apical membrane of epithelial cells (Ladias ,

2003; Wolde, 2007). Part of this regulation involves activated PKCε which binds to RACK1 and translocates to the plasma membrane. RACK1 interacts directly with NHERF1 by PDZ domain interaction at the apical membrane of epithelial cells. It is hypothesized that the complex composed of PKCε – RACK1-NHERF1 interacts with CFTR to regulate its membrane stability (Fig. 3 & 4).

7. Conclusions

Although VIP binding to the $VPAC_2$ receptor plays an important role in the relaxation of smooth muscles and is a matter of intense study for respiratory diseases such as bronchial asthma (Hamidi *et al*, 2006; Alessandrini *et al*, 1993; Groneberg *et al*, 2001; Groneberg *et al*, 2006; Jaeger *et al*, 1996; Onoue *et al*, 2004; Said, 1991a), our recent studies have highlighted the important role of the $VPAC_1$ receptor and differential activation of the PKA or PKC signalling pathways in airway epithelial cells to regulate CFTR-dependent secretions. *In vivo* data set VIP, or its analogs, as a potential candidate for the treatment of CF as it corrects many features of this disease including the molecular basis. Further investigation of VIP potential to rescue exocrine epithelial secretions should be conducted to uncover the large potential of this peptide in the treatment of respiratory diseases, which are the third cause of hospitalization and death in North America.

8. Footnotes

1. Airway defense in response to acute stress such as intense exercise is under the control of the vagal nerve pathway (see Kubin, 2006 and Wine, 2007 for review).
2. G proteins are composed of 3 subunits: α, β and γ. They mediate signaling cascades initiated by ligand binding to membrane receptors of the G protein coupled receptors (GPCR) family. Once activated, the α subunit dissociates from βγ and translocates to a target effector: cellular enzymes and ion channels. The α subunits, which mediate most of the known signals, comprise 4 different types which will initiate specific signaling cascades: α_s, α_q, α_i, $\alpha_{12/13}$. The α_s subunit's effector is the adenylyl cylcase and it initiates the cAMP signaling cascade. The α_q subunit rather activates phospolipase C and initiates the calcium and PKC signaling cascades (for review see Musnier, 2010).

9. Acknowledgments

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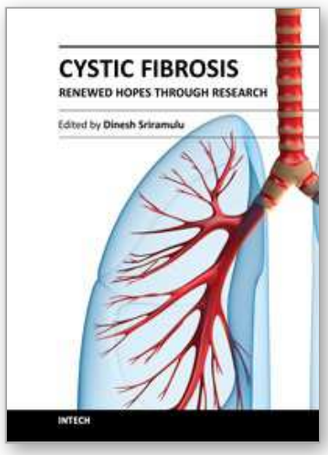

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Living healthy is all one wants, but the genetics behind creation of every human is different. As a curse or human agony, some are born with congenital defects in their menu of the genome. Just one has to live with that! The complexity of cystic fibrosis condition, which is rather a slow-killer, affects various organ systems of the human body complicating further with secondary infections. That's what makes the disease so puzzling for which scientists around the world are trying to understand better and to find a cure. Though they narrowed down to a single target gene, the tentacles of the disease reach many unknown corners of the human body. Decades of scientific research in the field of chronic illnesses like this one surely increased the level of life expectancy. This book is the compilation of interesting chapters contributed by eminent interdisciplinary scientists around the world trying to make the life of cystic fibrosis patients better.

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