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Outwardly Rectifying Chloride Channels and CF: A Divorce and Remarriage

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Outwardly rectifying Cl^- channels were originally thought to be the central element in cystic fibrosis. The role of these channels in CF was questioned to such an extent that doubts were raised about the validity of the original experiments. Recent data reestablishes a role for outwardly rectifying Cl^- channels (ORCC) in CF and suggests that the protein encoded by the CF gene, the cystic fibrosis transmembrane regulator (CFTR), can effect the regulation of more than one channel in the airway. This minireview deals with the rise, fall, and resurrection of the role of outwardly rectifying Cl^- channels in CF.

KEY WORDS: Chloride channels; CF; outwardly rectifying chloride channels; *c*AMP, CFTR, review.

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease which is common in North America (Collins, 1992). The hallmarks of the disease include thick, dehydrated airway mucus, chronic *Pseudomonas* lung infection, pancreatic insufficiency, bile duct obstruction, infertility in males, reduced fertility in females, high sweat Cl^-, intestinal obstruction, nasal polyp formation, and chronic sinusitis (Collins, 1992). Quinton (1983) suggested several years ago that Cl^- transport is defective in each of the tissues affected by CF. Since Quinton unified the thinking on the defect in CF, much attention has been given to understanding the biophysical properties, and the molecular and cellular biology of the Cl^- channels which generate Cl^- conductances in epithelial cells affected by CF. With the cloning of the CF gene the focus of many studies has been on the normal functioning of the CF gene product and how mutations in the CF gene influence the function of Cl^- channels. The first patch clamp studies on Cl^--secreting epithelia suggested that ORCCs generated Cl^- conductances in secretory epithelia. This class of channels was the center of much research and the source of much excitement because ORCCs were thought to be the critical cellular component that was defective in CF. Just prior to, and for some time following the identification of the CF gene, the role of outwardly rectifying Cl^- channels in CF was doubted and excitement and interest in this class of channels waned considerably. Discussed in this review are the unique biophysical properties of ORCCs, the evidence that caused many researchers to consider them the central component in CF, the subsequent doubts about their role in CF, and finally the evidence which reestablished their role in CF.

CHARACTERISTICS OF ORCCs

ORCCs (Fig. 1A) are a distinct class of Cl^- channels found in several human epithelial and non-epithelial tissues including human airway cells (either freshly isolated or grown in culture), colonic epithelial cells, pancreatic duct cells, sweat gland, and in lymphocytes (Gray *et al.*, 1989; Krouse *et al.*, 1989; Chen *et al.*, 1989; Frizzell *et al.*, 1986; Garber, 1992; Halm *et al.*, 1988; Hayslett *et al.*, 1987; Hwang *et al.*, 1989; Lin *et al.*, 1992; Solc and Wine, 1991). ORCCs have

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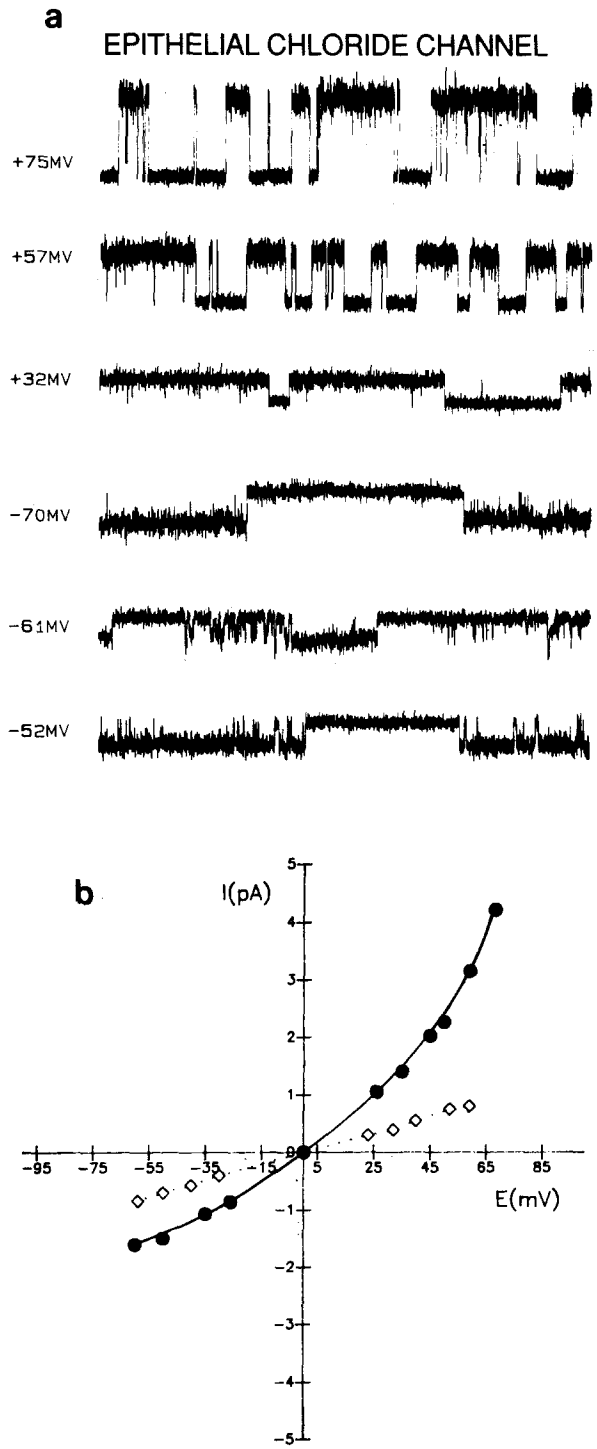


Fig. 1. A. Single-channel recording of ORCC activity measured in human airway cells. This channel was activated by depolarizing voltages. B. Current versus voltage relationships of ORCCs and SLCCs in human airway cells (IB3-1, CF bronchial epithelial cells transduced with a normal copy of CFTR). Measurements were made using excised inside-out patches. (●) ORCCs, (◇) SLCCs. Taken from Egan *et al.* (1992).

biophysical properties which give them a unique fingerprint in comparison to other types of Cl^- channels. For example, the anion selectivity is I > Cl > Br (see Egan *et al.*, 1992). Consistent with their name, ORCCs have a nonlinear current versus voltage relationship in symmetrical Cl^- solutions (Fig 1B) such that the conductance is higher when current is flowing out of the cell ($\approx$ 40–50 pS) than when Cl^- flows into the cell ($\approx$ 19 pS). For a negative ion like Cl^-, this means that the channel itself is asymmetrical in allowing Cl^- to flow more easily into the cell. In secretory epithelial cells, Cl^- channels are usually involved in the net movement of Cl^- out of the cell. The molecular basis and functional role of the rectification are unknown.

The gating behavior of channel activity involves an inactive state in which channel activity is absent, and an active state in which short and long openings and closings are abundant (see Fig. 1A). The channels at rest are in the inactive state and must be stimulated to enter the active state. A broad range of compounds and physical maneuvers can activate these channels. Protein kinases such as *c*AMP-dependent protein kinase A (PKA) and protein kinase C (PKC) are thought to be physiological stimulators of channel activity (see Welsh *et al.*, 1989), but a host of nonphysiological factors can function as activators. For example, when patches of membrane which contain inactive ORCCs are excised at 22°C and at −50 mV (transmembrane potential with pipette grounded), there is normally no spontaneous channel activity. However, when initially silent patches are exposed for seconds to minutes to strongly depolarizing voltages > +50 mV (see Hwang *et al.*, 1989), ORCCs will enter the active state. Exposure to these voltages appears to jolt the channel into a prolonged active state. Moderate changes in voltage do not reverse the process. For example, channels initially inactive upon patch excision at −50 mV, when exposed to depolarizing voltages, remain active when the voltage is returned back to −50 mV. Inactive channels can also be stimulated by exposing patches to high salt concentrations, to trypsin, and, in some instances, by increasing bath temperatures from 22 to 37°C (see Welsh *et al.*, 1989 and Kunzelmann *et al.*, 1989).

Although the molecular basis for the pattern of ORCC gating is unknown, based on information on channels in excitable cells (see Hoshi *et al.*, 1990 and Zagotta *et al.*, 1990), it is likely that the transition from inactive to active states involves the dissociation either of a portion of a single protein or a subunit of a multimeric channel. This component of the channel

protein probably occludes the channel pore in the inactive state. Both high salt and increased temperatures would promote disassociation. Trypsin activates channels without an effect on channel conductance and thus may cleave the amino acid residues at the channel mouth involved in gating between inactive and active states (Welsh *et al.*, 1989). The structure of this trypsin-sensitive gating mechanism could involve basic amino acids which weakly sense the electric field. This may explain why only a very strong depolarization is effective in activating the channels. Likewise, physiological stimulators, such as PKA and PKC, may phosphorylate either the gating mechanism or some associated portion involved in allowing the channel to change state.

Irrespective of the mode of activation, the kinetics of the active state appear the same, with bursts of openings during which the open channel is interrupted only by short closings. These bursts are interspersed with longer closing events which range from seconds to minutes (Fig. 1A). No physiological regulators have been identified which regulate the open state.

There is also very little known about what regulates the transition from active to inactive states. Strong hyperpolarizations to > -80 mV reverse activation either induced by depolarizing voltages or raised temperature. This gives further strength to the argument that the gating mechanism weakly senses the electric field (Welsh *et al.*, 1989).

With regards to physiological inactivators, it may be expected that if phosphorylation is involved in activation, then a phosphatase should mediate inactivation. However, it has never been demonstrated that phosphatases can indeed inactivate ORCCs.

As mentioned, PKC can activate ORCCs (Hwang *et al.*, 1989; Li *et al.*, 1989). However, this ability appears to depend upon the concentration Ca^{2+} in the solution bathing the intracellular face of the membrane. ORCCs are not intrinsically Ca^{2+} sensitive. For example, changing bath Ca^{2+} does not activate channels or alter the kinetics of channels already in the active state. Although not affecting channel gating directly, high Ca^{2+} (> 150 nM), on the intracellular face of the membrane, can reverse the effect of PKC (Li *et al.*, 1989). At high Ca^{2+}, PKC actually inhibits channel activity. Thus, PKC probably plays a dual role in modulating channel activity. Gating of ORCCs probably involves several molecular steps with both stimulatory and inhibitory phosphorylation sites.

Ion channels are often characterized by their sensitivity to various blocking agents. ORCCs are blocked by several classes of compounds including stilbene, carboxylic acid, indanyloxy alkanoic acid derivatives, and some commonly used pH buffers (Hanrahan and Tabcharani, 1990; Hayslet *et al.*, 1987; Hwang *et al.*, 1990; Kunzelmann *et al.*, 1989; Singh *et al.*, 1991). Each of these compounds causes a "flickery-type" of interruption in the open channel current when applied to either face of the membrane. In addition to the more well-known inhibitors such as DIDS (4,4′-diisothiocyanostilbene-2,2′-disulfonic acid) and NPPB (5-nitro-2-[3-phenylpropylamino]-benzoic acid), other compounds such as arachidonic acid, leukotrienes, and other *cis*-unsaturated fatty acids, such as linoleic acid and the laxative, ricinoleic acid, also cause brief interruptions in open channel currents (Anderson and Welsh, 1990; Hwang *et al.*, 1990). The effect is evident at micromolar concentrations of the fatty acids, with 25 μM arachidonic acid decreasing the open time of the channel by 10-fold. The interaction of the fatty acids with the channel is complex (Hwang *et al.*, 1992). Often channel blockers operate by plugging the pore of an open channel. However, the effects of voltage and bath Cl^- concentrations on the off rates and on rates of fatty acid-induced channel interruptions suggest that these compounds do not behave as simple charged blockers of an open ORCC. Rather than simply plugging the pore, the interaction of fatty acids may influence channel gating (Hwang *et al.*, 1992). If fatty acids do indeed alter channel gating, then it is possible that fatty acids are interacting with a site on the channel which modulates channel gating and that natural modulators of channel activity control channel gating by binding to this site. There is some evidence that a natural modulator of ORCCs may exist in the cytosol of several epithelia involved in Cl^- secretion. For example, exposing ORCCs to cytosol from secretory epithelia inhibits channel activity with high potency (Krick *et al.*, 1991; Kunzelmann *et al.*, 1991). The exact nature of this cytosolic component and its physiological role are unknown. The identification of a cytosolic inhibitor is intriguing because it also raises the possibility that protein kinases, depolarizing voltages, and high salt and temperature may all activate this channel by inducing the removal of a cytosolic inhibitor.

The majority of studies discussed up to this point have attempted to determine how particular second messenger systems interact with ORCCs. A few studies have focused on the external receptors involved

in activating ORCCs. These studies have implicated extracytosolic ATP, via a purinergic, P_2 receptor in the apical cell membrane of airway epithelial cells as a candidate for a hormonal activator of ORCCs (Stutts *et al.*, 1992). For example, ATP applied to apical solution of airway epithelia stimulates Cl^- secretion. The stimulation occurs in cells containing both normal and mutant forms of CFTR. It is thought to happen through a CFTR-independent pathway because stimulation can be seen in mouse airway cells known to be completely lacking CFTR expression (Clarke *et al.*, 1992). Application of 100 μM ATP to the extracellular face of an excised patch increases open probability by about 300% suggesting that the increase in Cl^- secretion in intact epithelia may occur via the stimulation of ORCCs (Stutts *et al.*, 1992). Interestingly, activation by ATP does not occur through the most common second messenger agonists such as Ca^{2+}, G proteins, *c*AMP, or PKC. This raises the intriguing possibility that the P_2 receptor is either part of the channel or intimately associated with the channel. These studies are particularly important for CF research because of the potential that ATP activation of ORCCs could be used as a therapy which would increase the amount of fluid in the airways and enhance pulmonary clearance.

Clearly, a great deal of information is available which demonstrates that outwardly rectifying Cl^- channels can be defined by a unique set of biophysical properties, stimulators, and inhibitors. Many questions regarding the physical basis of the gating and the interaction of the various inhibitors with either the gating mechanism or the channel pore remain unanswered. Most of this information will be easier to obtain once the molecular biology of the channel is known.

PRE-CFTR HISTORY OF ORCCs

Quinton's (1983) discovery of defective Cl^- conductances in epithelia from CF patients came at a time when the patch clamp technique was beginning to be applied to epithelial tissues. The use of the patch clamp technique made it possible to define the properties of ion channels which generate epithelial ionic conductances. Much attention was given to understanding the nature of the channels involved in Cl^- secretion (Frizzell *et al.*, 1986a). The first study of apical Cl^- channels in human tracheal epithelial cells was reported by Welsh (1986). In cell-attached patches, he observed apical Cl^- channels which were activated by isoproterenol, a classic agonist of Cl^- secretion in airway epithelia. In excised patches the channels were outwardly rectifying and blocked by analogs of carboxylic acid which were known to block other types of epithelial Cl^- channels (Oberleithner *et al.*, 1983). Based on these early experiments, it was suggested that ORCCs are responsible for the apical Cl^- conductance in airway epithelia. Subsequently, it was shown by Frizzell *et al.* (1986b) that β-adrenergic agonists stimulated outwardly rectifying, apical cell membrane Cl^- channel activity in cell-attached patches of normal airway epithelial cells. The same agonists, however, failed to induce Cl^- channel activity in airway cells from CF patients. Similar observations were made by Welsh and Liedke (1986). These were important observations because they firmly linked CF to defective regulation of an apical Cl^- channel. The question remained open, however, whether the channel itself was defective or whether some component of the second messenger pathways was not operating properly.

To examine the process of activation and the defect in CF, several groups performed experiments on excised patches to show that purified PKA could activate ORCCs in normal airway cells. These studies showed that PKA failed to activate ORCCs in cells from CF patients (Li *et al.*, 1988; Schoumacher *et al.*, 1987). Depolarization activation verified that channels were indeed present but failed to be activated following PKA treatment. Not only were channels present in CF cells, but their basic biophysical properties (single-channel conductance, ionic selectivity) were identical to those from normal cells. The implication of these key experiments was that mutations in the CF gene cause subtle changes in the activation process interfering with the ability of kinases to phosphorylate the channel. If, on the other hand, phosphorylation did occur, then it was postulated that mutations somehow interfered with the gating mechanism which allowed the channel to enter the open state. The work of several investigators demonstrated convincingly that the defect in CF was primarily at the level of the channel or a closely associated regulatory protein and not in the second messenger systems involved in channel activation.

The role of ORCCs in CF was expanded when they were observed in both T and B lymphocytes. Surprisingly, ORCCs were shown to be activated by PKA only if the cells were taken from normal individuals. Again, in cells from CF patients, PKA failed

to activate the channels (Chen *et al.*, 1989). These data were surprising because involvement of lymphocytes in the primary defect in CF had not been shown prior to these experiments (Hagiwara *et al.*, 1989).

Excitement was raised even further when it was demonstrated in excised patches that PKC at Ca^{2+} concentrations within physiological levels for resting Ca^{2+} could activate channels obtained from normal human airway cell and that PKC could not activate channels excised from CF patients (Hwang *et al.*, 1989 and Li *et al.*, 1989). This implicated a second kinase in the regulation of ORCCs and suggested that perhaps a second phosphorylation site was defective in CF, or more likely that transduction of information from phosphorylation sites to the gating mechanism was not operating properly.

Given this overwhelming evidence from several laboratories, it was strongly felt that the basic defect in CF was defective regulation of ORCCs. Prior to the sequencing of the gene, many researchers postulated that the CF gene would encode an ORCC or a closely associated regulatory protein.

THE DIVORCE

At about the time when excitement and interest in ORCCs were most intense, data were beginning to be generated that raised doubts on the involvement of ORCC both in epithelial Cl^- secretion and in CF. One early study (Gray *et al.*, 1989) showed that small, linear conductance, channels (SLCC) with conductances between 4–7 pS were present in high numbers in pancreatic duct cells. In contrast, ORCCs were observed only infrequently. This suggested that in pancreas, which is dysfunctional in CF, perhaps SLCCs were responsible for net Cl^- secretion and not ORCCs. Furthermore, the activity of SLCCs was increased up to three-fold in cell-attached patches by exposing pancreatic duct cells to secretin, dibutyryl cyclic AMP, or forskolin. Thus, SLCCs were not only more abundant, but were also under hormonal control via *c*AMP-dependent second messenger system. No work on CF pancreas was reported so the implications for CF research were unclear.

More serious doubts about ORCCs were raised when the whole cell patch clamp approach, which measures the currents and conductances generated across both apical and basolateral cell membranes of epithelial cells instead of single channels in a small patch of membrane, began to be utilized to assess the Cl^- currents generated by Cl^--secreting epithelia. Cliff and Frizzell (1990) were the first to show that the Cl^- conductance, activated by forskolin and *c*AMP in T_{84} cells, has a linear current versus voltage relationship and an absence of voltage dependence. The properties of this *c*AMP-dependent current were more like those of the low-conductance channel identified in pancreatic epithelial cells and not consistent with ORCC generated currents. Although CF cells were not studied directly, it also suggested that another channel may be defective in CF.

Table I. Properties of SLCC vs ORCC

	SLCC	ORCC
I/V	Linear	Rectifying
Conductance	4–13 pS	≈45 pS
DIDS	Insensitive (500 μM)	Sensitive (50 μM)
Rundown	Yes	No
Selectivity	Br > Cl	I > Cl

The sequencing of the CF gene, information on the CFTR protein, and expression of the CF gene in experimental cell systems further eroded the faith of the scientific community in the role of ORCCs in CF (Riordan *et al.*, 1989; Rommens *et al.*, 1989). The CF gene was shown to encode a protein, CFTR, which is homologous to a family of ATP-binding cassette (ABC) proteins. Prior to the identification of CFTR, ABC proteins were not known to function as channels. CFTR was originally thought to be a conductance regulator which could perhaps transport a key substance necessary for the proper regulation of ion channels (Riordan *et al.*, 1989). However, it soon became apparent by expressing CFTR in experimental cell systems such as COS cells, insect SF9 cells, and *Xenopus* oocytes, that CFTR can function as a Cl^- channel with properties distinctly different from the outward rectifier (see Table I and Anderson *et al.*, 1991a,b; Bear *et al.*, 1991, 1992; Drumm *et al.*, 1990, 1992; Gregory *et al.*, 1990; Kartner *et al.*, 1991). The properties of CFTR (see Fig. 1B) were very similar to the small linear conductance channels (SLCC) observed in the pancreatic duct cells (Gray *et al.*, 1989), and to the *c*AMP-activated, linear whole cell currents detected in T84 cells (Cliff and Frizzell, 1990). Even stronger evidence that CFTR can function as a SLCC and that it is involved in defective Cl^- transport in CF came from an analysis of the effects of mutations in the CF gene on channel function. It was shown that mutations in the transmembrane domain

at position 95 could alter SLCC anion selectivity (Anderson *et al.*, 1991a). In addition, the most common mutation observed in CF patients, the ΔF508 mutation, causes a dramatic reduction in the open probability of the SLCC (Dalemans, 1992). This highly convincing evidence that CFTR can operate as a SLCC and that its properties are altered by mutations in the CF gene raised very serious doubts about the role of ORCC in CF. More serious doubts about ORCCs were raised by two reports which failed to observe PKA activation of ORCC in excised patches (Tabcharani and Hanrahan, 1991; Solc and Wine, 1991).

DO KINASES ACTUALLY REGULATE ORCCs

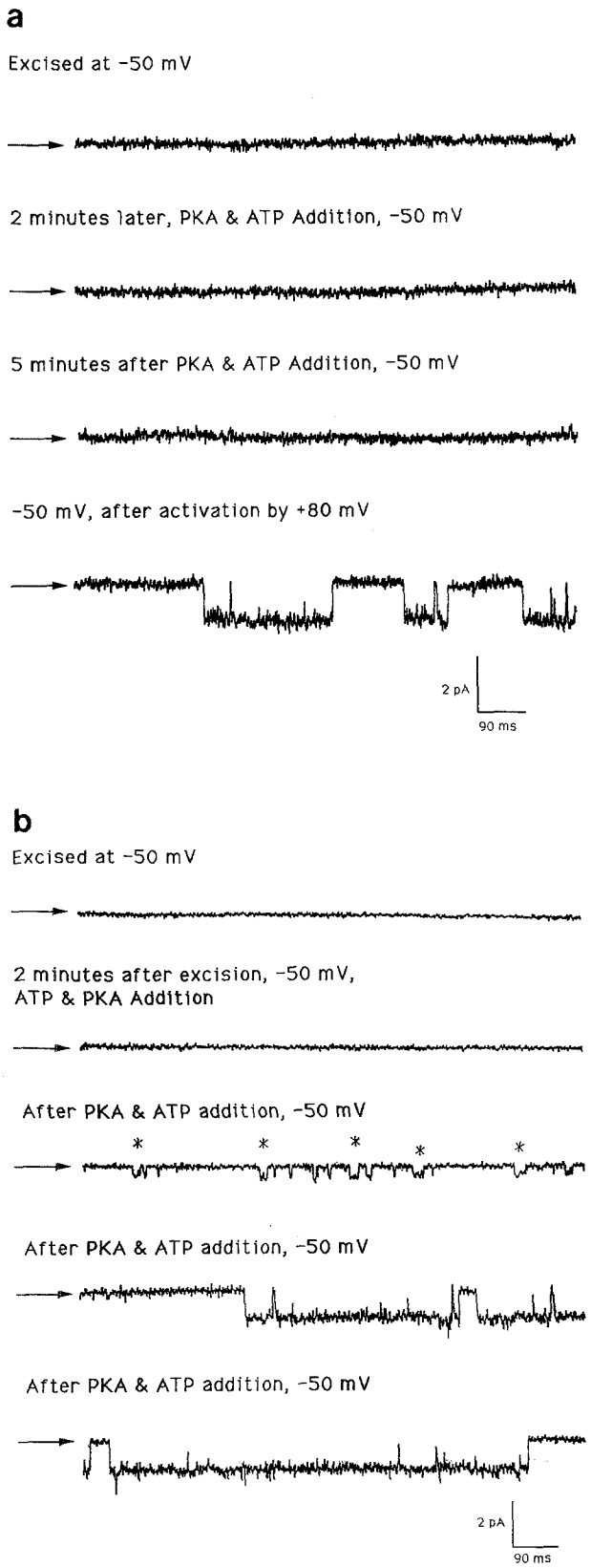
Because of the doubts raised about kinase regulation of ORCCs and their role in CF, it was important to examine carefully the experimental approach used to determine the activation of ORCCs by PKA and PKC. The experimental approach involved excising a patch of apical membrane at −50 mV and 22°C from a cell containing ORCCs, ascertaining that channels were not spontaneously active, applying PKC and ATP to the intracellular face of the patch, and waiting for active channels to appear (see Hwang *et al.*, 1989). Although experiments showing that PKA could activate ORCCs were carried out carefully by several different laboratories, it was suggested that during the procedure of excising a patch or adding kinases and other reagents to the bath, outwardly rectifying Cl^- channels could be activated inadvertently (Tabcharani and Hanrahan, 1991). This would mean that PKA does not really activate ORCCs and that the activation was an artifact of the experimental protocol.

Although it was possible that activation was inadvertent, it was extremely unlikely for the following reasons. First, spontaneous activation caused solely by patch excision does not readily occur when patches are excised at 22°C and at −50 mV (see Egan *et al.*, 1992). Second, in order for PKA activation to occur, both kinase and ATP must be present; addition of either PKA or ATP by themselves does not induce activation. Third, PKA and ATP when added in the presence of the Walsh PKA inhibitor, a peptide which inhibits the action of PKA, do not induce activation. Fourth, PKC activation does not occur when either ATP and PKC or diacyglycerol (DAG) and PKC are added. Activation occurs only when all three components are added together, indicating that not only is ATP required but the enzyme itself must be activated by DAG to be effective in evoking channel activity. Fifth, activation in the presence of PKC, DAG, and ATP can be inhibited by staurosporine (see Hwang *et al.*, 1989). All of these experiments provide convincing evidence that an ATP-dependent phosphorylation reaction is involved in activating ORCCs. The failure to observe activation either when no stimulants are added, when components are added individually, or added together but in the presence of an inhibitor eliminate the possibility of mistaking inadvertent activation for PKA or PKC activation.

THE REMARRIAGE: A LINK BETWEEN CFTR AND THE REGULATION OF ORCCs

Given this evidence that kinases activate ORCCs and the activation is not an artifact of the experimental protocols, then the question is, how does the old data on defective regulation of ORCCs in CF fit with newer evidence that CFTR functions as a SLCC? A likely explanation is that somehow mutations in the CF gene are having an effect on more than one channel. If it is true that there is a relationship between CFTR and ORCCs, then it follows that inserting a normal copy of the CF gene into CF cells should correct defective regulation of ORCCs. Figure 2A shows an excised patch clamp experiment performed on a CF bronchial epithelia cell line (IB3-1, Zeitlin *et al.*, 1991). ORCCs are present in the parent IB3-1 cells, but PKA fails to activate them. Stably transfecting these CF cells with a normal copy of CFTR, using an adeno-associated viral vector (AAV), corrects defective Cl^- secretion (see Egan *et al.*, 1992). In the CF cell line stably transfected with a normal copy of CFTR (S9 cells), both ORCCs and SLCCs become active when excised patches are treated with PKA and ATP (see Fig. 2B). This demonstrates that insertion of a normal copy of the CF gene into a cell line derived from a CF patient corrects defective regulation of ORCCs and induces the expression of small-conductance, linear channels. These experiments suggest that CFTR can effect the regulation of more than one channel and reestablish the involvement of ORCCs in CF.

The question is, how does CFTR control PKA activation of outwardly rectifying Cl^- channels? Several scenarios are possible. One intriguing possibility is that the CF gene also encodes two different types of channels, perhaps by alternate splicing of mRNA.



This may be unlikely for several reasons. First, CF gene expression does not appear to correlate with the presence of outwardly rectifying Cl^- channels (Ward *et al.*, 1991). Second, CFTR introduced into experimental cell systems such as COS cells, SF9 cells, and *Xenopus* oocytes expresses a small, linear-conductance Cl^- channel with blocker sensitivities and ionic selectivities very different from the larger channel (Anderson *et al.*, 1991a,b; Bear *et al.*, 1991, 1992; Drumm *et al.*, 1990, 1992; Gregory *et al.*, 1990; Kartner *et al.*, 1991). Another compelling possibility is that since CF is associated with defective acidification of intracellular organelles, it may induce PKA regulation by affecting outwardly rectifying Cl^- channels as they are processed within the intracellular organelles (Barasch *et al.*, 1991). Still another possibility is that CFTR may transport a substance which allows activation of outwardly rectifying Cl^- channels. This transported substance may actually be ATP. Recently, there have been some preliminary data that CFTR may also transport ATP out of the cells (Reisin *et al.*, 1991). ATP can activate ORCCs in both normal and CF cells via a purinergic receptor on the extracellular surface of airway epithelia (Stutts *et al.*, 1992). Thus, if CFTR transports ATP, it could in turn activate ORCCs in an autocrine fashion.

Clearly there is growing evidence that CFTR may have more than one function. In addition to functioning as chloride channel, CFTR may, as was originally thought, act as a conductance regulator. There is precedence for a duality of function in ABC proteins, with the observations that the multidrug resistance transporter can function both as a transporter of hydrophobic drugs and as a volume-sensitive Cl^- channel (Valverde *et al.*, 1992). The same duality of function is likely to be evident for CFTR.

The hypothesis that CFTR can have more than one function is consistent with the symptoms of CF in humans which are expressed in several functional modes in airway including enhanced Na^+ reabsorption,

Fig. 2. A. Chloride channels in CF bronchial epithelial cells (CFBE). Excised patches from IB3-1 parent cells were held at −50 mV for 2 min before 50 nM PKA and 1 nM Mg-ATP were added to the cytoplasmic side of the membrane. The failure of PKA activation in this patch was monitored for an additional 5 min. Presence of outwardly rectifying chloride channels was verified by voltage activation. B. PKA activation of low-conductance and ORCCs in cells transfected with a normal copy of CFTR. Patches excised from S9 cells and held at −50 mV for at least 2 min before PKA and Mg-ATP were added. * indicates low-conductance channel activity. Taken from Egan *et al.* (1992).

abnormal processing and trafficking of proteins and membrane vesicles, and chronic *Pseudomonas* infection (see Collins, 1992). These abnormalities occur in addition to the well-documented defect in *c*AMP-induced Cl^- secretion and are important because 90% of the deaths in CF occur from repeated lung infection brought about by viscous mucus secretions and not directly from defective Cl^- channel function (see Collins, 1992). Therefore, determining how CFTR interacts with these functional modes will be important for the development of therapeutic strategies.

Concerning ORCCs, several questions remain unanswered. One of the most important is to define their physiological role in the different cell types where they are found. Do they play a role in Cl^- secretion or perhaps volume regulation? Equally important for the CF research is to know precisely how CFTR interacts with ORCCs to allow kinase activation. Finally, most important for the CF patient will be the prospect that activation of ORCCs may provide an alternative pathway for Cl^- secretion.

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