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cAMP Activation of CF-Affected Cl^- Conductance in Both Cell Membranes of an Absorptive Epithelium

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Summary. Cystic fibrosis (CF) is characterized by abnormal epithelial Cl^- conductance (G_{Cl}). In vitro studies that have shown that cAMP regulation is an intrinsic property of the CF-affected G_{Cl}(CF-G_{Cl}) have been carried out previously on cultured secretory cells and on nonepithelial cells. Even though G_{Cl} in absorption is defective in CF, a clear demonstration of cAMP regulation of CF-G_{Cl} in a purely absorptive tissue is lacking. We studied the cAMP regulation of CF-G_{Cl} in the microperfused intact human reabsorptive sweat duct. About 40% of the ducts responded to cAMP (responsive) while the remainder of the ducts did not. In responsive ducts, cAMP-elevating agents: β-adrenergic agonist isoproterenol (IPR), CPT-cAMP, forskolin, theophylline or IBMX increased G_t by about 2.3-fold (n = no. of ducts = 8). Removal of media Cl^-, but not amiloride pretreatment (in the lumen), abolished the cAMP response, indicating exclusive activation of G_{Cl}. cAMP activated both apical and basolateral G_{Cl}. cAMP hyperpolarized gluconate : Cl^- (lumen : bath) transepithelial bionic potentials (ΔV_t = -20.3 ± 5.2 mV, mean ± SE, n = 9) and transepithelial 3 : 1 luminal NaCl dilution diffusion potentials (ΔV_t = -8.8 ± 2.9 mV, n = 5). cAMP activated basolateral G_{Cl} as indicated by increased bi-ionic (gluconate : Cl^-, bath : lumen) diffusion potentials (by about 12 mV). The voltage divider ratio in symmetric NaCl solutions increased by 60%. Compared to responsive ducts, nonresponsive ducts were characterized by smaller spontaneous transepithelial potentials in symmetrical Ringer's solution (V_t = -6.9 ± 0.8 mV, n = 24, nonresponsive *vs.* -19.4 ± 1.8 mV, n = 22, responsive ducts) but larger bi-ionic potentials (-94 ± 6 mV, n = 35, nonresponsive *vs.* -65 ± 5 mV, n = 17, responsive ducts) and dilution diffusion potentials (-40 ± 5 mV, n = 11, nonresponsive *vs.* -29 ± 3 mV, n = 7, responsive ducts). These results are consistent with an inherently (prestimulus) maximal activation of G_{Cl} in nonresponsive ducts and submaximal activation of G_{Cl} in responsive ducts. We conclude that cAMP activates CF-G_{Cl} which is expressed and abnormal in both apical and basal membranes of this absorptive epithelium in CF.

Key Words CFTR · regulation · sweat duct · cystic fibrosis

Introduction

The gene product responsible for cystic fibrosis (CF)[1] appears to be a defective Cl^- channel [1, 6, 12]. Recent evidence indicates that cAMP regulation is an intrinsic property of CF-Cl^- channels which is greatly reduced in CF [1, 3, 6, 12], but most of this evidence of cAMP regulation of CF-Cl^- channels has been derived from cultured secretory cells [3, 11, 33] and from nonepithelial cell lines transfected with the CF gene [1, 6, 12]. However, CF-affected Cl^- channels are clearly expressed in reabsorptive as well as secretory epithelial functions. The genetic nature of the disease implies that cAMP regulation may be an intrinsic property of CF-affected Cl^- channels in reabsorptive function as well. Therefore, a better understanding of cAMP regulation of CF-G_{Cl} in an "intact epithelium" with a "purely absorptive" function may help to link these important in vitro observations more closely to the pathology of CF [17, 19].

The human sweat duct offers unique opportunities to investigate the properties of regulation of CF-G_{Cl} in an absorptive epithelium. The sweat duct has an exclusively NaCl absorptive function and its transepithelial G_{Cl} (about 80% of tissue conductance) is almost entirely comprised of CF-G_{Cl} [5, 15, 18]. The sweat duct is one of the very few human epithelial tissues readily accessible for the study of CF-G_{Cl} in an intact epithelium. Moreover, secretory cells do not apparently express large Cl^- conduc-

[1] *Abbreviations:* CF = cystic fibrosis; G_t = transepithelial conductance; V_b = electrical potential across the basolateral membrane; V_a = electrical potential across the apical membrane; V_t = transepithelial potential; ΔV_b = transepithelial current-induced voltage deflections across the basolateral membrane; ΔV_a = transepithelial current-induced voltage deflections across the apical membrane; ΔV_t = transepithelial current-induced voltage deflection across the epithelium; VDR = voltage divider ratio; G_{Cl} = transepithelial Cl^- conductance; CF-G_{Cl} = cystic fibrosis-affected Cl^- conductance; EMF = electromotive force; IPR = isoproterenol; IBMX = 3-isobutyl-1-methylxanthine; CPT-cAMP = chlorophenylthio-adenosine 3′-5′ cyclic monophosphate; PGE_2 = prostaglandin E_2.

tances in the basolateral membrane so that only the apical membrane G_{Cl} has been shown to be defective in CF-affected secretory epithelia [3, 37]. However, in the reabsorptive sweat duct, Cl^- is transported across both the apical and basolateral membranes via conductive Cl^- channels down its electrochemical gradient [24, 25–27]. We recently showed preliminary evidence that the G_{Cl} at both the apical and the basolateral membranes are abnormal in the sweat duct [25, 27]. These observations raise the question of the involvement of basolateral G_{Cl} in the absorptive CF epithelia. Two possible explanations for these results could include: (i) Cl^- impermeability in the basolateral membrane of reabsorptive tissues may be a secondary consequence of a primary lesion in the apical membrane, or (ii) CF-Cl^- channels may be involved in Cl^- transport across both cell membranes of sweat duct. If CF-Cl^- channels are expressed in both cell membranes, the G_{Cl} at apical and basolateral membranes should have similar properties of regulation while being consistent with the properties of CF-Cl^- channels expressed in secretory cells.

In support of the second possibility, we present evidence herein which indicates that (i) cAMP can activate CF-G_{Cl} in reabsorptive function in a manner similar to observations in secretory epithelial cells and CF gene transfected nonepithelial cells [2, 3, 6, 7, 11, 23, 37], and (ii) the G_{Cl} at the basolateral membrane as well as at the apical membrane is regulated by cAMP and is abnormal in CF.

Materials and Methods

Tissue Acquisition

Sweat glands were obtained as previously described from adult male volunteers giving informed consent. Briefly, 3-mm diameter full-thickness biopsies were taken from the back over the scapula and stored overnight in cold Ringer's solution with or without collagenase (2 mg/ml). Individual sweat glands were removed in cold Ringer's solution (*see below*) by dissection with fine-tipped tweezers visualized at a magnification of ×80. The isolated glands were transferred to a dissecting stage cooled with a Peltier block to 4°C in the same basic salt solution and further dissected into its components of reabsorptive sweat duct and a secretory coil. Segments of ~1 mm or longer of sweat ducts were separated from the coiled portion of the gland and transferred to a perfusion chamber for microcannulation. The duct was microperfused and perifused with appropriate solutions as required. Manipulations and measurements were made at 36 ± 1°C.

Solutions

The standard Ringer's solution contained (in mM): 150 NaCl, 2.13 K_2HPO_4, 0.38 KH_2PO_4, 1.0 $MgSO_4$, 1.0 calcium acetate, and 10 glucose at pH 7.4. In Cl^--free Ringer's solution, gluconate (150 mM) was used to replace Cl^-. In 50 mM NaCl, mannitol was generally used to balance the solution's osmolality, although such changes in osmolality of luminal solution had no detectable effect on electrical properties due to the water impermeability of the apical membrane. As required 0.1 mM amiloride was added to the luminal solutions. Isoproterenol (IPR, 10^{-5} M), propranolol (10^{-5} M), forskolin (10^{-6} M), 3-isobutyl-1-methylxanthine (IBMX, 10^{-6} M), chlorophenylthio-adenosine 3′-5′cyclic monophosphate (CPT-cAMP, 10^{-4} M), cholinergic agonist mecholyl (10^{-6} M), antagonist atropine (10^{-6} M), prostaglandin-E_2 (PGE_2, 10^{-6} M), and cyclooxygenase inhibitor indomethacin (10^{-6} M) were added to the bath solutions. Since all cAMP-elevating agents had similar effects on the electrical properties of sweat ducts [21], we grouped the data from all these agonists under cAMP responses.

Electrical Mesurements

After cannulating the lumen of the isolated sweat ducts with a double-lumen cannula made from theta glass (1.5 mm diameter; Clark Electromedical Instruments, Reading, UK), a constant current pulse of 1.0–0.5 sec and 50–100 nA was injected through one barrel of the cannulating pipette containing NaCl Ringer's solution. The other barrel of the cannulating pipette served as an electrode for measuring the transepithelial potential difference (V_t) with respect to the contraluminal bath and as a cannula for perfusing the lumen of the duct with selected solutions. V_t was monitored continuously using one channel of a WPI-700 dual electrometer referenced to the contraluminal bath.

Microelectrodes were pulled from Clark glass capillaries using a Brown-Flaming microelectrode puller (model P-77). The microelectrodes were filled with 4 M K^+ acetate. Electrode resistance was 50–100 MΩ, and tip potentials were <4 mV. A piezo electric hybrid manipulator, PM 500–20 Frankenberger (Munich, FRG), mounted on an X-Y manipulating base was used to impale the cells.

Intracellular potentials were measured using a Dagan single-electrode voltage-clamp amplifier (model 8100-1). Basolateral membrane potential (V_b) was measured with reference to contraluminal bath ground. Apical membrane potential (V_a) was calculated as the difference between V_b and V_t. The criteria for accepting the results from impaled cells were described earlier [24].

The voltage divider ratio (VDR) of apical-to-basolateral membrane resistances was measured using the voltage deflections induced by transepithelial constant-current pulse. The basolateral membrane voltage deflection (ΔV_b) was measured directly with the impaling microelectrode. The apical membrane voltage deflection (ΔV_a) was determined by subtracting ΔV_b from ΔV_t. The VDR was taken as $\Delta V_a/\Delta V_b$, which is equal to the ratio of the resistance of the apical membrane to the resistance of the basolateral membrane.

K^+-Sensitive Microelectrodes

The procedures for the fabrication and measurements of intracellular potassium activity in the sweat ducts are the same as described earlier by Reddy and Quinton [28].

Statistics

Results were calculated as the mean ± SE with n equal to the number of ducts (for transepithelial measurements) and cells (for intracellular potentials and VDR measurements) from a minimum

of three human subjects. The Student's *t* test was used to evaluate statistical significance.

Results

Variable Effects of cAMP

Application of agents which increase intracellular cAMP such as IPR, CPT-cAMP, forskolin, theophylline, and IBMX caused a detectable increase in G_t in about 40% of the ducts. If a duct was sensitive to one agent, it was sensitive to all agents. Likewise, if a duct did not respond to one agent, it did not respond to any agonist. The remainder of ducts (60%) did not respond to any of the cAMP-elevating drugs. Based on the presence and absence of detectable electrical response to any of the cAMP-elevating drugs, we retrospectively divided sweat ducts into "cAMP-responsive" and "cAMP-nonresponsive" ducts.

Effect of cAMP on the Electrical Properties of Responsive Ducts

General

Application of IPR, CPT-cAMP, forskolin, theophylline, or IBMX induced similar electrophysiological responses at low concentrations (10^{-6} to 10^{-5} M). The effects of these drugs were reversible, although the time course and extent of reversal of response upon the washout of the drugs was variable. The reversal from cAMP stimulation was only partial in some ducts, such that the response to subsequent stimulation seemed blunted. The bioelectric changes described below refer to cAMP-responsive ducts. None of these changes were observed in nonresponsive ducts.

Basal Electrical Properties

In responsive ducts, cAMP decreased V_b and V_t in symmetrical 150 mM NaCl (Fig. 1*A*, *B*; Table 1). The lack of effect of cAMP on V_b and V_t is correlated with either low baseline values or application of cAMP more than once (Fig. 1*B*). The cAMP effects on V_b and V_t were more pronounced in the presence of Cl^- gradients (50 mM NaCl in the lumen) as indicated by a significant hyperpolarization of V_b and V_t (Fig. 2; Table 1). The changes in V_a were generally much smaller.

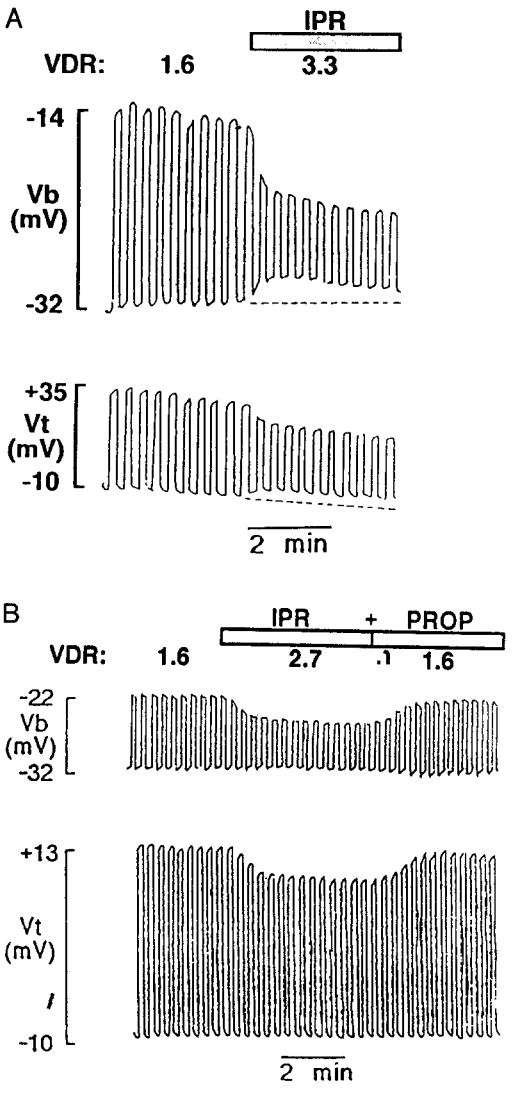

Fig. 1. (*A*) Effect of IPR on the basal electrical properties (V_t and V_b), current-induced voltage deflections (ΔV_t and ΔV_b) and VDR. Note that forskolin depolarized V_t and V_b, increased G_t and VDR and decreased ΔV_b, consistent with the activation of G_{Cl} at the basolateral membrane. (*B*) Effect of second application of IPR, nearly 2 hr after initial stimulation on the basal electrical properties (V_t and V_b), current-induced voltage deflections (ΔV_t and ΔV_b) and VDR in the same cell as in *A*. Note that the ΔV_t and ΔV_b are still smaller, as compared to the prestimulated values in *A*, indicating only a partial recovery from the first application (*A*). Further, IPR had little effect on V_t and V_b, despite a small increase in the VDR and G_t (decrease in ΔV_t). Comparison of *A* and *B* indicates that reversal of activated G_{Cl} to the inactivated state may take a long time (up to several hours), which might explain the lack of cAMP responsiveness in some ducts. Also note that the effect of IPR is reversed by the β-adrenergic antagonist propranolol.

G_t

cAMP significantly increased the G_t from about 30 to 70 mS (Fig. 3). cAMP had no effect on G_t in Cl^--

Table 1. Effect of cAMP on the electrical properties of responsive ducts

	Control	cAMP	ΔV_t
150 NaCl (L) / 150 NaCl (B) (n = 22)	−19.4 ± 1.8	−14.3 ± 1.8[a]	+5.9 ± 0.8
150 Na gluconate (L) / 150 NaCl (B) (n = 9)	−32.8 ± 7.8	−53.1 ± 7.1[a]	−20.3 ± 5.2
50 NaCl (L) / 150 NaCl (B) (n = 5)	−29.4 ± 4.1	−38.2 ± 6.8[a]	−8.8 ± 2.9

cAMP decreased spontaneous (150 NaCl, L) but increased bionic (150 Na gluconate, L) and dilution (50 NaCl, L) diffusion potentials of responsive ducts, indicating an increase in G_{Cl}. ΔV_t = difference in the baseline V_t between control and cAMP-stimulated ducts. Values of V_t given in mV are mean ± SE "n" refers to the number of ducts from a minimum of five subjects.
[a] Significantly different from control ($P < 0.05$).

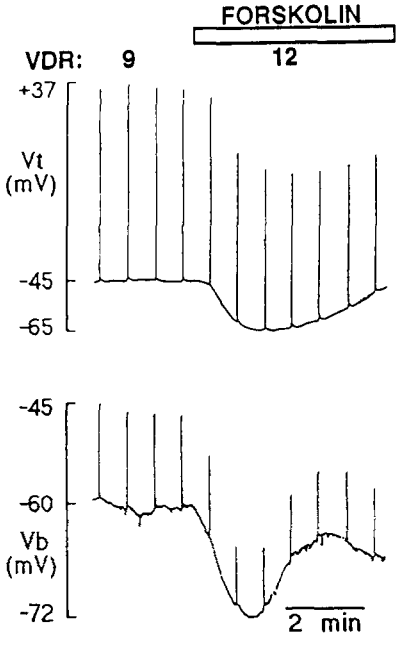

Fig. 2. Effect of forskolin on the basal electrical properties (V_t and V_b) and VDR in the presence of imposed Cl^- gradients (50/150 mM NaCl, lumen/bath). Note that cAMP stimulation had greater effects on V_t and V_b in the presence of larger Cl^- gradients (*compare* Fig. 1*B*). Also note that forskolin increased VDR even in low-luminal NaCl concentrations, indicating that the cAMP-activated basolateral G_{Cl} is fairly uniform over a wide range of luminal salt concentrations as generally experienced by the distal portions of the duct.

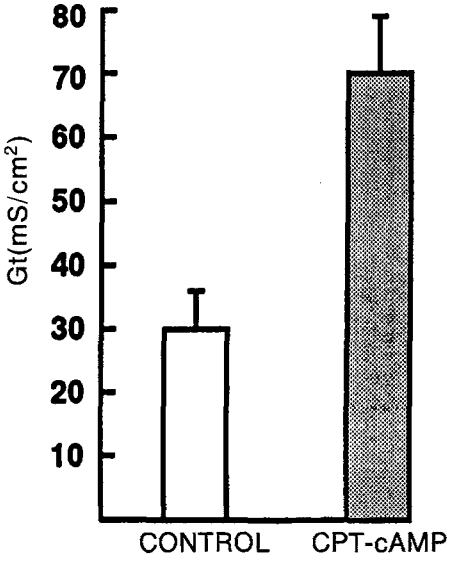

Fig. 3. Effect of cAMP on the G_t of responsive ducts in 50/150 mM NaCl lumen/bath. Note that cAMP increased G_t by two- to threefold.

free medium (n = 4), even though the response persisted after the inhibition of sodium conductance by luminal amiloride (Fig. 4).

Bi-Ionic Gradients

cAMP increased the bi-ionic diffusion potential (generated by complete luminal Cl^- substitution with the impermeant anion gluconate; Table 1; Fig. 5), while simultaneously decreasing current pulse-induced transepithelial voltage deflections (ΔV_t) from 41.4 ± 5.5 to 31.7 ± 5.1 mV (n = 3). We did not see any significant changes in luminal diameter due to cAMP stimulation, suggesting that the decrease in ΔV_t is due to an increase in G_t. Preliminary evidence suggests

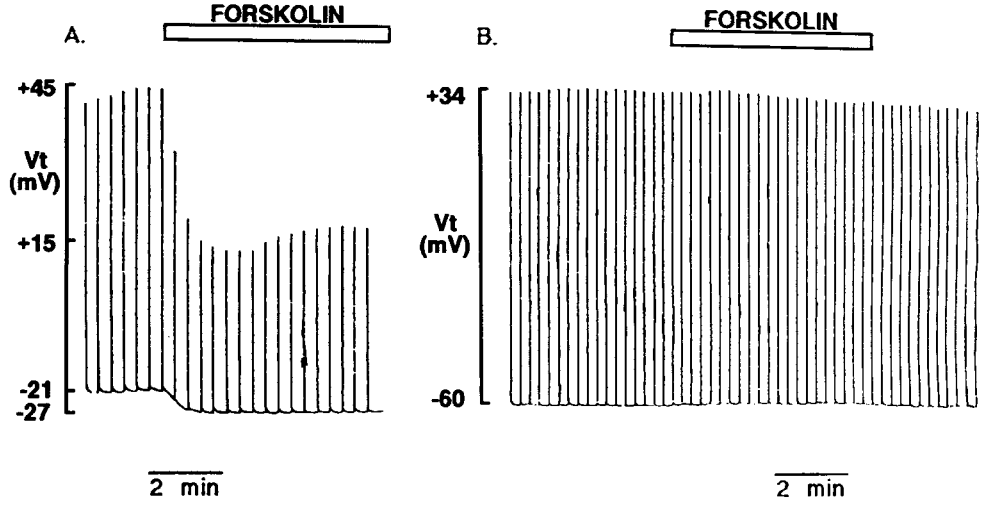

Fig. 4. Effect of forskolin on V_t and G_t (as indicated by constant-current pulse-induced transepithelial voltage deflections, ΔV_t): With 50 mM NaCl + amiloride in the lumen and 150 mM NaCl in the bath, forskolin increased V_t and G_t (*A*). However, in Cl^--free medium (with an open G_{Na}, i.e., no amiloride in the lumen), forskolin had no effect on V_t and G_t, indicating that forskolin primarily increases G_{Cl} without affecting the cation conductances (*B*). The lumen diameter remained constant throughout all manipulations.

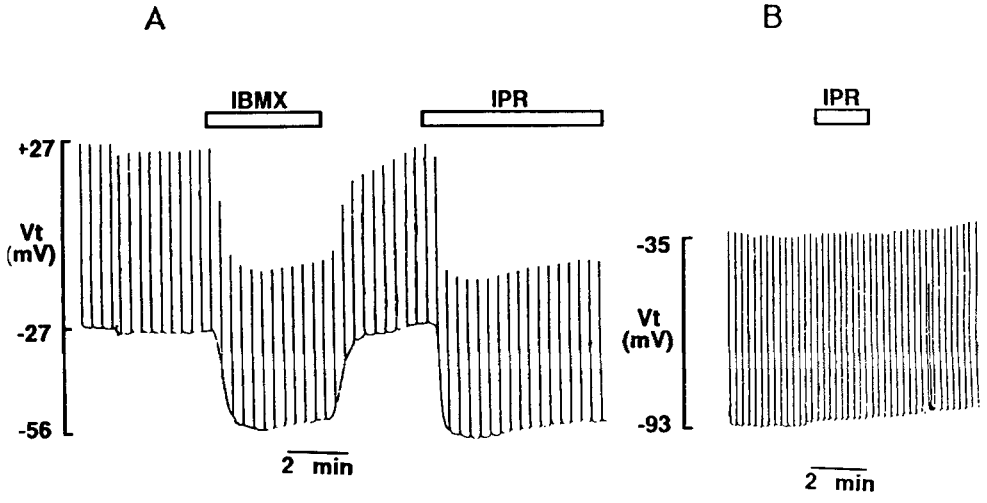

Fig. 5. Comparison of effect of either IBMX and IPR on the bi-ionic Cl^- diffusion potentials in sweat ducts with low (*A*) and high (*B*) initial bi-ionic Cl^- diffusion potentials. Note that IBMX and IPR induced qualitatively and quantitatively similar increases in bi-ionic diffusion potentials and G_t (as indicated by decreased amplitude of ΔV_t) in the low (*A*) but IPR had no effect in the high (*B*) potential duct.

that cAMP also increased the reversed bi-ionic potentials generated by complete serosal bath Cl^- substitution while significantly decreasing ΔV_t (Fig. 6).

Dilution Diffusion Potentials

cAMP also increased the amplitude of dilution diffusion potentials while simultaneously decreasing current-induced ΔV_t from 69.1 ± 5.3 to 57.2 ± 5.9 mV (n = 5). The increase in diffusion potentials ranged from −4 to −20 mV (Table 1; Fig. 7).

VDR

cAMP increased the VDR of responsive ducts in 150 mM symmetric NaCl (Fig. 1*A* and *B*; Table 2), in 1:3 luminal dilute solution (Fig. 2; Table 3), and in luminal amiloride (Table 4). The cAMP caused a

Table 2. Effect of cAMP on current-induced voltage deflection and VDR of responsive ducts

	ΔV_t	ΔV_b	ΔV_a	VDR
150 NaCl (L) / 150 NaCl (B)	38.3 ± 8.5	11.7 ± 1.9	26.6 ± 7.3	2.3 ± 0.5
150 NaCl (L) / 150 NaCl + cAMP (B)	32.2 ± 8.6	6.4 ± 0.6[a]	25.7 ± 8.1	3.8 ± 0.9
(n = 5)				

cAMP increased VDR and decreased ΔV_t and ΔV_b. ΔV_t, ΔV_b and ΔV_a are voltage deflections in response to 100-nA transepithelial current pulses. Values in mV are mean ± SE. "n" refers to the number of cells from a minimum of three subjects.
[a] Significantly different from control ($P < 0.05$).

Table 3. Effect of cAMP on current-induced voltage deflections and VDR of responsive ducts in dilute luminal salt solution

	ΔV_t	ΔV_b	ΔV_a	VDR
50 NaCl(L) / 150 NaCl(B)	68.7 ± 4.9	11.0 ± 1.8	57.7 ± 6.3	5.6 ± 1.6
50 NaCl(L) / 150 NaCl + cAMP(B)	50.7 ± 5.8[a]	6.2 ± 0.7[a]	45.1 ± 6.3	7.7 ± 2.2
(n = 3)				

cAMP increased VDR and decreased the amplitudes of ΔV_t, ΔV_b and ΔV_a in dilute luminal NaCl (50 mM). ΔV_t, ΔV_b and ΔV_a are voltage deflections in response to 100-nA transepithelial current pulses. Values in mV are mean ± SE. "n" refers to the number of cells from a minimum of three subjects.
[a] Significantly different from control ($P < 0.05$).

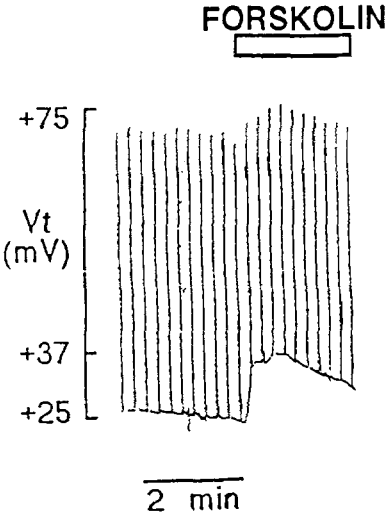

Fig. 6. Effect of forskolin on the reversed bi-ionic Cl^- diffusion potentials generated by complete bath Cl^- substitution in the bath while microperfusing the lumen with 150 mM NaCl Ringer's. Forskolin increased the positive bi-ionic potentials consistent with activation of G_{Cl} in the basolateral membrane.

decrease in both ΔV_b and ΔV_a, but ΔV_b decreased more than ΔV_a (Figs. 1 and 2; Tables 2–4).

Intracellular Potassium Activity

IPR did not have any effect on the intracellular potassium activity. The intracellular potassium activity remained at about 92.5 ± 13.2 mM before and during the application of IPR. Even though, there was about 48-mV outwardly directed driving force for the exit of potassium across the basolateral membrane (V_b = −27.5 ± 3.0 mV, n = 4 cells).

Comparison of Spontaneous Electrical Properties of cAMP-Nonresponsive and -Responsive Ducts

In order to find out the physiological basis of cAMP nonresponsiveness we divided the ducts into two groups, "cAMP-responsive" and "cAMP-nonresponsive" ducts based on the presence and absence of response to cAMP. We compared the spontaneous electrical properties of these two groups of ducts (prior to stimulation).

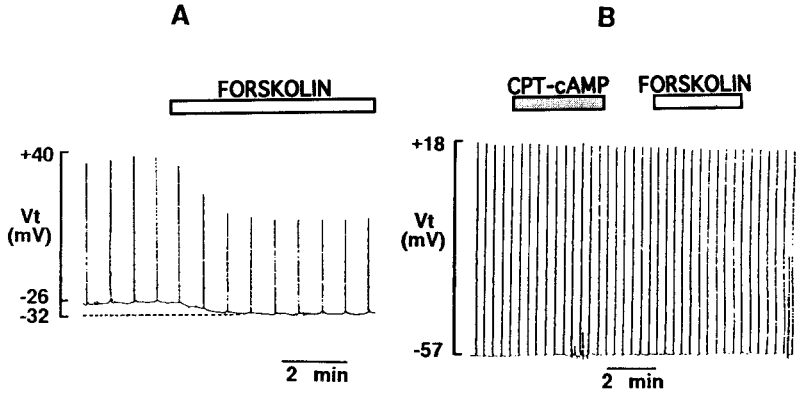

Fig. 7. Comparison of effect of forskolin on the dilution diffusion potentials in sweat ducts with low (*A*) and high (*B*) initial diffusion potentials. Forskolin increased V_t and G_t (decreased ΔV_t) in low (*A*) but not high (*B*) potential duct.

Table 4. Effect of cAMP on current-induced voltage deflection and VDR in amiloride-inhibited responsive ducts

	ΔV_t	ΔV_b	ΔV_a	VDR
150 NaCl + Amil(L) / 150 NaCl(B)	37.3 ± 1.9	7.0 ± 1.8	30.0 ± 1.2	5.0 ± 1.0
50 NaCl + Amil(L) / 150 NaCl + cAMP(B)	33.0 ± 1.1[a]	5.2 ± 1.0	27.6 ± 0.4	5.8 ± 0.9

Amiloride (10^{-5} M) was added to luminal perfusion solution to eliminate Na conductance from any cAMP responses. ΔV_t, ΔV_b and ΔV_a are voltage deflections in response to 100-nA transepithelial current pulse. Values in mV are mean ± SE. Four measurements were made on ducts from three different subjects.

[a] Significantly different from control ($P < 0.05$).

V_t

Before application of any agonist, the mean V_t (−7 mV) of nonresponsive ducts was significantly lower ($P < 0.05$) in symmetrical (150 mM) NaCl concentration (Fig. 8; Table 5) than in responsive ducts (−19 mV), although the ranges of V_t overlapped considerably between nonresponsive (−1 to −16 mV) and responsive ducts (−7.8 to −34 mV).

Bi-Ionic Diffusion Potentials

Before stimulation, the mean of bi-ionic diffusion potentials generated by complete luminal Cl^- substitution by gluconate was significantly larger in nonresponsive ducts (−94 mV) as compared to responsive ducts (−65 mV) (Fig. 5; Table 5). Likewise, the ranges of bi-ionic diffusion potentials also overlapped considerably for nonresponsive (−70 to −135 mV) and responsive ducts (−40 to −105 mV).

Dilution Diffusion Potentials

Even though the ranges of dilution diffusion potentials completely overlapped (−20 to −80 mV for nonresponsive ducts and −23 to −45 mV for responsive ducts), the means of dilution diffusion potentials before cAMP stimulation were significantly larger in nonresponsive ducts (−40 mV) as compared to the responsive ducts (−29 mV) (Fig. 7; Table 5).

VDR

Before cAMP stimulation, the mean VDR of nonresponsive ducts was larger (3.6 ± 0.8, $n = 5$, $P < 0.05$) than that of responsive ducts (2.3 ± 0.4, $n = 5$). However, the VDR of responsive ducts appears similar to that of nonresponsive ducts after stimulation with cAMP (3.8 ± 0.9, $n = 5$, Fig. 9).

Potential Regulatory Agents

β-Adrenergic

The electrophysiological effects of β-adrenergic agonist IPR were similar to those induced by cAMP-elevating agents (Fig. 1*A* and *B*). The effect of IPR was reversed by the β-adrenergic antagonist propranolol. Acute or overnight incubation of the nonresponsive

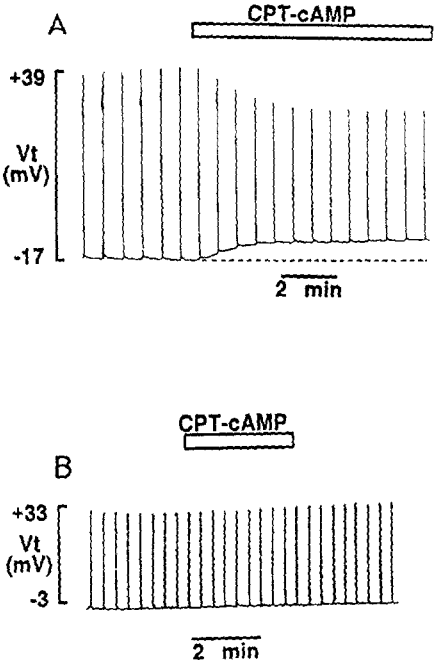

Fig. 8. Comparison of effect of CPT-cAMP on the spontaneous V_t (150 mM NaCl in lumen and bath) and G_t (as indicated by ΔV_t) of sweat ducts with high (*A*) and low (*B*) V_t. cAMP depolarized V_t and increased G_t in the duct with high (*A*), but not low (*B*) V_t, consistent with submaximal and maximal activation of G_{Cl} in high and low V_t ducts, respectively.

ducts in propranolol (10^{-5} M) did not induce cAMP responsiveness upon washing out of propranolol.

Cholinergic

The cholinergic agonist mecholyl had no effect on the electrical properties of either responsive or nonresponsive ducts (Fig. 10). Overnight incubation of ducts with muscarinic cholinergic antagonist atropine (10^{-6} M) did not affect the basal electrical properties or the susceptibility to cAMP stimulation of the ducts.

PGE_2

PGE_2 evoked electrophysiological responses in sweat ducts similar to cAMP-elevating drugs. In nonresponsive ducts, PGE_2 had no effect on G_t and V_t (Fig. 11), whereas in responsive ducts, PGE_2 evoked qualitatively identical electrophysiological responses as those induced by cAMP-elevating agents (Fig. 10). However, either acute or overnight incubation of the ducts in the cyclooxygenase inhibitor indomethacin (10^{-6} M) neither affected the basal electrical properties nor induced cAMP responsiveness in nonresponsive ducts upon washout of the drug.

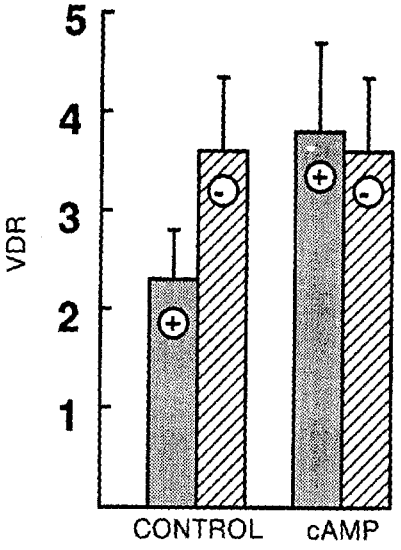

Fig. 9. Comparison of effect of cAMP on the VDR of responsive (+) and nonresponsive (−) ducts. VDR of nonresponsive ducts was smaller as compared to the responsive ducts. However, upon stimulation, the VDR of responsive ducts increased to values comparable to the VDR of nonresponsive ducts (which did not change upon stimulation), suggesting that G_{Cl} increased in the basolateral membrane more in responsive than in nonresponsive ducts.

Discussion

Is the cAMP Effect on Absorption Physiological?

Since cAMP is known to increase the G_t in the secretory epithelia [35], we followed Cl^- conductance properties as simple, direct measure of cAMP regulation of ion transport in sweat duct. Our results indicated that about 40% of the ducts examined responded to agents known to elevate intracellular cAMP by increasing G_t (Fig. 3). The fact that we did not see a cAMP response in a majority of the ducts raised concern as to the relevance of cAMP effects in the physiology of sweat ducts. Several lines of evidence strongly suggest that cAMP is an intracellular messenger for regulation of ion transport in this tissue as well as in many other epithelia [8, 11, 33, 36]. First, elevation of intracellular cAMP (by addition of its analog CPT-cAMP, activation of adenylate cyclase by forskolin, inhibition of phosphodiesterase by IBMX or theophylline) evoked similar electrophysiological responses [21], suggesting that the mechanism of action of these drugs is mediated by a shared intracellular messenger, most probably cAMP (*see below*). Second, duct cells in primary culture also respond to cAMP [4, 29], indicating that sweat duct cells in fact inherently possess mecha-

Table 5. Spontaneous electrical properties of responsive and nonresponsive ducts

	150 NaCl	150 NaGlu	50 NaCl
Responsive ducts	−19.4 ± 1.8 (n = 22)	−65.2 ± 4.8 (n = 17)	−28.6 ± 3.0 (n = 7)
Nonresponsive ducts	−6.9[a] ± 0.8 (n = 24)	−94.3[a] ± 5.9 (n = 35)	−40.1[a] ± 5.3 (n = 11)

The spontaneous electrical properties of responsive and nonresponsive ducts are significantly different. Both groups of ducts were microperfused with 150 mM NaCl (150 Cl), 150 mM NaGlu (150 Glu), or 50 mM NaCl (50 Cl) while being perifused with 150 mM NaCl Ringer's. The transepithelial voltages (V_t) in all the three conditions indicate that nonresponsive ducts are inherently more permeable to Cl^- than responsive ducts. Values of V_t given in mV are mean ± SE. "n" refers to the number of ducts from at least five subjects.

[a] Significantly different from responsive ducts ($P < 0.05$).

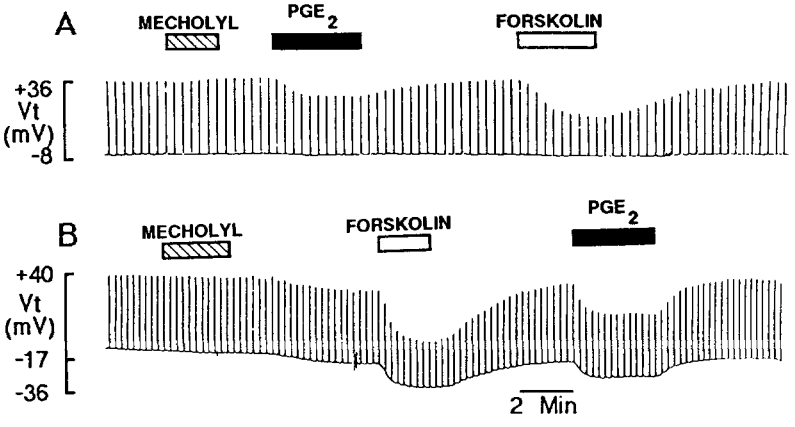

Fig. 10. Effect of mecholyl, PGE_2 and forskolin on a responsive duct. Note that PGE_2 and forskolin evoked parallel electrical changes, whereas cholinergic stimulation had no effect. (*A*) Effect of PGE_2 and forskolin in symmetric NaCl concentration in lumen and bath. (*B*) Effect of PGE_2 and forskolin (50 mM NaCl lumen/150 mM NaCl bath) on dilution diffusion potentials.

nisms for cAMP regulation of G_{Cl}. Third, the G_{Cl} of ducts responsive to cAMP appeared to be submaximal prior to cAMP stimulation (Table 5; Figs. 4, 5, 7 and 8), whereas the G_{Cl} of nonresponsive ducts appeared to be maximally activated by endogenous mechanisms prior to any stimulation. These differences in the activation of G_{Cl} responsiveness of sweat ducts reflect a difference in the initial status of G_{Cl} (for details, *see below*) and is apparently inherent to transport regulation.

Which Ionic Conductance Is Regulated by cAMP

Our results indicate that cAMP primarily activates G_{Cl}, without detectable effects on either sodium conductance in the apical membrane or potassium conductance in the basolateral membrane [24, 28]. In Cl^--free medium cAMP had no effect on the electrical properties of the duct (Fig. 4). If cAMP were to activate potassium or sodium conductance we should have seen an increase in G_t. In the absence of sodium conductance (amiloride in the lumen), cAMP increased both G_t (Fig. 4) and VDR (Table 4) of normal ducts. Further, β-adrenergic stimulation, which elevates intracellular cAMP, had no detectable effect on apparent potassium conductance, since intracellular potassium activity did not change, even though there was an enormous gradient for potassium exit (~40 mV; ref. [28]). These results suggest that the predominant, if not the only, conductance regulated by cAMP in the sweat duct is G_{Cl}. Although we cannot rule out at this time cAMP effect on tight-junction permeability, overwhelming evidence from several epithelial studies shows that a cAMP-regulated G_{Cl} resides in cell membranes [11, 16, 31, 35, 38]. In the sweat duct, we do not know if the cAMP-regulated G_{Cl} is located in the apical or basolateral membrane, so we sought to determine the site (apical and/or basolateral membranes) of expression of cAMP-dependent G_{Cl} in sweat duct.

cAMP Activates a G_{Cl} in the Apical Membrane

In order to determine if cAMP activates G_{Cl} in the apical membrane, we studied the effect of cAMP on

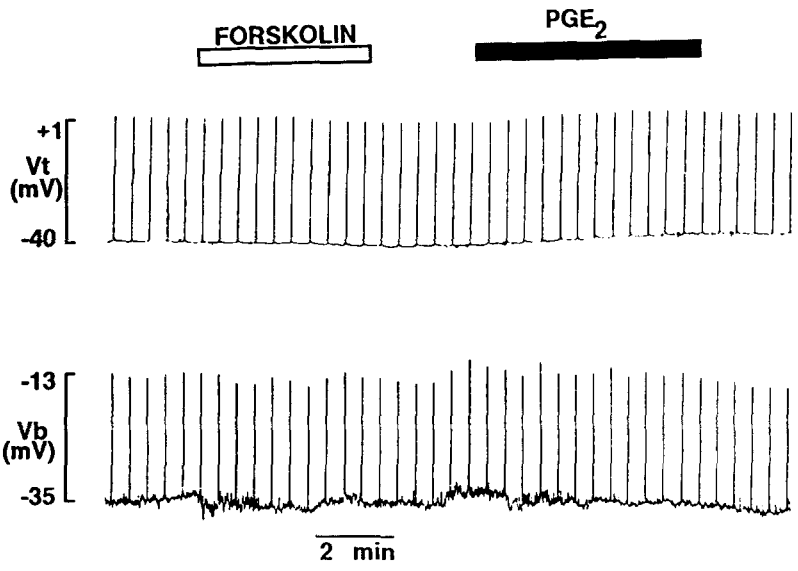

Fig. 11. Lack of effect of PGE_2 on a nonresponsive duct (50 mM NaCl lumen/150 mM NaCl bath). Note that PGE_2 has no effect on V_t, V_b and G_t in nonresponsive ducts.

transepithelial bi-ionic and dilution diffusion potentials. Activation of an apical G_{Cl} should cause an increase in the amplitude of bi-ionic and dilution diffusion potentials, which was observed immediately after application of cAMP in responsive ducts (Figs. 5 and 7; Table 1). In addition, if cAMP were to activate apical G_{Cl}, we should also see a decrease in the voltage drops across the apical membrane (ΔV_a) induced by transepithelial current pulses. As expected, ΔV_a decreased in the presence of cAMP which is more pronounced in low-luminal NaCl (50 mM) concentration (Tables 2–4).

These observations are consistent with the previous report that the apical membrane of sweat ducts possess Cl^- channels with conductance properties similar to those described in other tissues [15]. Our results are also consistent with the observations on several other epithelial cells which showed that cAMP regulates epithelial G_{Cl} at the apical membrane [6, 8, 35]. The fact that the G_{Cl} at the apical membrane of CF ducts is also abnormal in CF [25, 27] as is the cAMP-regulated G_{Cl} at the apical membrane of secretory cells argues that the G_{Cl} in the apical membrane [25, 27] of sweat ducts is also regulated by cAMP [2, 12, 37].

In the secretory epithelium, only G_{Cl} at the apical membrane appears to be activated by cAMP because the G_{Cl} at the basolateral membrane of the secretory tissues is relatively insignificant [4, 19, 37]. In sweat ducts, a G_{Cl} exists in both apical and basolateral membranes of normal ducts and is abnormal in both membranes of CF ducts [25, 27]. The abnormality in the G_{Cl} of the basolateral membrane could be either due to the simultaneous expression of CF-Cl^- channels in the basolateral and apical membranes or due to a secondary consequence of a primary defect at the apical membrane. If the first possibility is correct, we expect similar regulation of G_{Cl} in both membranes, assuming that the CF gene product is a Cl^- channel [1, 6, 12]. We therefore asked whether cAMP regulates G_{Cl} in the basolateral membrane of the sweat duct.

cAMP Activates G_{Cl} at the Basolateral Membrane

Several of our observations indicate that cAMP activates a G_{Cl} in the basolateral membrane of sweat ducts. In responsive ducts, cAMP increased the positive bi-ionic diffusion potentials generated by complete Cl^- substitution in the bath (Fig. 6). Since an increase in the bi-ionic diffusion potential can also be expected if cAMP activates G_{Cl} in the tight junctions (due to the introduction of Cl^- EMF across Cl^--selective paracellular pathway [24]), we studied the effect of cAMP on intracellular potentials, voltage drops across the cell membranes as a function of transepithelial constant-current pulses, and the VDR. We found that cAMP depolarized the basolateral membrane and V_t in responsive ducts (Figs. 1*A* and 8), indicating an increase in the relative Cl^- conductance in the basolateral membrane [24–28]. However, cAMP effect(s) on V_b and V_t could be too small to be detected during second application [Fig. 1*A* (first application) and Fig. 1*B* (second application)], or in ducts with relatively smaller V_b and V_t, suggesting that the cell potential is close to the Cl^- EMF [24–28]. Further, after imposing a luminal Cl^- gradient, cAMP hyperpolarized V_b and V_t (Fig. 2),

suggesting that the lack of effect of cAMP on V_b and V_t of some responsive ducts in symmetric NaCl solutions in lumen and bath is due to the near equilibrium distribution of Cl^- across the cell membranes [4, 28, 29].

More significantly, cAMP decreased ΔV_b (Fig. 1*A* and *B*; Tables 2–4). If cAMP had not increased the basolateral membrane conductance we should have seen an increase in ΔV_b due to an increase in transcellular current corresponding to the cAMP activation of apical G_{Cl} (Figs. 5 and 6; Table 1). Simultaneously, cAMP significantly increased VDR (Figs. 1*A* and *B* and 2; Table 2). The increase in VDR was not due to a decrease in the apical sodium conductance because VDR also increased in the presence of amiloride (Table 4), nor was it due to an increase in the basolateral membrane potassium conductance because cAMP does not have a detectable effect on the potassium conductance in sweat duct (as discussed). In the absence of Cl^-, cAMP also failed to affect any of the observed electrical properties. Thus, perhaps surprisingly, the decrease in ΔV_b reveals a relatively larger increase in the G_{Cl} of the basolateral membrane (assuming that cAMP does not significantly increase the G_{Cl} at tight junctions, as discussed earlier).

cAMP-activated G_{Cl} in the basolateral membrane appears not to be unique to the sweat duct. cAMP-dependent Cl^- channels were also found in the basolateral membrane of medullary thick ascending limb [31, 38]. Although it is evident that cAMP activates G_{Cl} at both the cell membranes, the neurohumoral mechanisms responsible for the regulation of intracellular cAMP in the sweat duct are not known. Knowledge of neurohumoral control of cAMP in reabsorption seems to be essential not only to establish its physiological relevance but also to understand its role in CF pathology.

Factors Controlling Intracellular cAMP

Intracellular cAMP in the sweat duct appears to be under the control of β-adrenergic receptors, and possibly PGE_2, but it is not affected by cholinergic agonists (Fig. 10).

β-Adrenergic Control

β-adrenergic stimulation is known to increase intracellular cAMP in sweat gland cells [13]. Further, the β-adrenergic agonist IPR induced identical electrophysiological responses as other cAMP-elevating drugs (Fig. 5 and ref. [21]), confirming that β-adrenergic response is mediated by intracellular cAMP. The β-adrenergic antagonist propranolol reversed the effect of IPR, indicating that IPR response is mediated by β-adrenergic receptors. Additionally, the presence of adrenergic nerve terminals in the sweat gland [34] and the demonstration of β-adrenergic response in the cultured duct cells [29] further indicate β-adrenergic control of intracellular cAMP. However, intracellular cAMP may not be under the sole control of β-adrenergic receptors. The presence of prostaglandin-like activity in the human sweat [9, 10] which is associated with increases in intracellular cAMP levels [16, 30] and the demonstration of PGE_2-activated G_{Cl} in cultured duct cells [22] suggest that PGE_2 may also be involved in the control of cAMP. Until now, we had no direct evidence of PGE_2-regulated G_{Cl} in intact sweat duct. We therefore studied the effect of PGE_2 on G_t and Cl^- diffusion potentials on responsive and nonresponsive ducts.

PGE_2 Control

Our results suggest that intracellular cAMP is also under the control of PGE_2. PGE_2 evoked parallel electrophysiological responses in responsive ducts as with cAMP-elevating agents. In responsive ducts PGE_2 induced qualitatively similar changes in G_t and V_t and increased Cl^- diffusion potentials (Fig. 10), indicating the activation of G_{Cl} by PGE_2 similar to the cAMP-elevating agents. The fact that PGE_2 is known to increase intracellular cAMP [16, 30] coupled with the observation that PGE_2 and cAMP agents elicited similar electrophysiological responses in G_t and Cl^- diffusion potentials suggest that the action of PGE_2 involves an increase in the intracellular cAMP levels. The lack of effect of PGE_2 on nonresponsive ducts further suggest a convergence of the mechanisms of action of cAMP-elevating drugs and PGE_2 probably at the level of intracellular cAMP. It has been suggested that the G_{Cl} of cultured duct cells is primarily activated by PGE_2 and is insensitive to the activation of adenylate cyclase by forskolin [22] which contrasts with the present observations in the intact duct (Figs. 2, 4, 6, 7 and 10) as well as in cultured duct cells [4].

Physiological Basis of cAMP Nonresponsiveness

There are at least four possible explanations for cAMP nonresponsiveness of the ducts, which include (i) physical damage of nonresponsive ducts,

(ii) regional variations in physiological properties of proximal and distal portions of the ducts, (iii) variations among individual donors, and (iv) differences in the physiological status of different ducts depending on the levels of intracellular cAMP (and corresponding levels of activation of G_{Cl}) at the time of evaluation.

To assess potential physical damage of the nonresponsive ducts, we compared the Cl^- diffusion potentials (bi-ionic and dilution) of nonresponsive and responsive ducts. Cl^- diffusion potentials of nonresponsive ducts are larger than responsive ducts (Figs. 5 and 7; Table 5), not smaller as they would have been if they were damaged. We also compared the effect of cAMP on proximal (closer to the secretory coil) and distal (away from the secretory coil) ducts and found mixed responses to cAMP among proximal and distal ducts (*results not shown*). Variation among donors cannot explain cAMP nonresponsiveness either, since we found both responsive and nonresponsive ducts within the same plug of skin. Thus, our results are not consistent with any of the first three explanations.

In contrast, cAMP nonresponsiveness of some ducts appears to be due to a persistent maximal activation of G_{Cl} as indicated by Cl^- diffusion potentials and VDRs. A comparison of the electrical properties in the responsive and nonresponsive ducts indicated that the nonresponsive ducts have smaller spontaneous V_t and larger bi-ionic and dilution diffusion potentials (Figs. 5, 7 and 8; Table 5). These results reveal a higher G_{Cl} in nonresponsive ducts because a larger Cl^- shunt conductance attenuates the electrical potential generated by active sodium absorption and increases the relative contribution of Cl^- (EMF) diffusion potentials (Figs. 5, 7 and 8; Table 1). Changes in VDR showed that in responsive ducts, cAMP predominantly increased the G_{Cl} in the basolateral membrane (Figs. 1*A* and *B* and 2), while having no detectable effect on the G_{Cl} in the basolateral membrane of nonresponsive ducts (Fig. 9). If the lack of cAMP effect on the VDR of nonresponsive ducts is due to the prior activation of G_{Cl} at the basolateral membrane, we expect a larger VDR in nonresponsive ducts as compared to the responsive ducts (because the G_{Cl} and the overall basolateral membrane conductance of nonresponsive ducts would be expected to be larger as compared to those of responsive ducts). In fact, the VDR of nonresponsive ducts was significantly larger than that of responsive ducts (Fig. 9). cAMP stimulation increased the apparent G_{Cl} at the basolateral membrane and VDR of responsive ducts to similar levels observed in nonresponsive ducts. However, cAMP had no effect on either the VDR or any of the current-induced voltage deflections in nonresponsive ducts (Fig. 9; *see* Results). These observations suggest that the basal G_{Cl} of basolateral membrane is initially (prior to stimulation) larger in nonresponsive ducts as compared to responsive ducts. Interestingly, a few ducts which were initially nonresponsive became responsive to cAMP when the transepithelial conductance fell over time during microperfusion [21], lending further support to the notion that cAMP responsiveness depends on the level of initial activation of G_{Cl}.

Phosphorylation *vs.* Dephosphorylation in the Activation of G_{Cl}

Studies on the mechanisms of regulation of epithelial G_{Cl} suggested a two-step control process: (i) activation of the channel protein by kinase-dependent phosphorylation and (ii) inactivation of the channel by phosphatase-dependent dephosphorylation [6, 14, 32]. We surmise that the prolonged maximal activation of G_{Cl} in most of our duct preparations is either due to the persistently high levels of intracellular cAMP or to the weak or low phosphatase activity. If the persistent activation of G_{Cl} in nonresponsive ducts is due to the high levels of cAMP, we should be able to inhibit the G_{Cl} by reducing the levels of intracellular cAMP [22, 30]. We attempted to reduce intracellular cAMP levels by inhibiting the two most important sources of cAMP production (the β-adrenergic receptor activation and PGE_2) by application of either β-adrenergic antagonist propranolol or cyclooxygenase inhibitor indomethacin [22, 30]. Overnight or acute incubation of nonresponsive ducts either in propranol or in indomethacin did not decrease G_{Cl} nor induce cAMP responsiveness after washout of these drugs (*results not shown*). In contrast, acute application of propranolol reversed the effect of IPR in responsive ducts (Fig. 1*B*), suggesting that there may be a steady-state balance between kinase activation and phosphatase inhibition of G_{Cl}- in responsive ducts. Once Cl^- channels are activated by cAMP-dependent phosphorylation, the inactivation of G_{Cl} may involve, but not be limited to, the rate of dephosphorylation. Phosphatase activity in nonresponsive ducts may be very low, leading to persistent activation of G_{Cl} in nonresponsive ducts.

Nature of cAMP-Regulated G_{Cl} in Sweat Ducts

The transepithelial G_{Cl} in the sweat duct is comprised almost entirely of CF-affected Cl^- channels. The G_{Cl} (which contributes 80% to the G_t) in sweat

duct is completely inhibited in CF [5, 18]. The G_{Cl} of both apical and basolateral membranes of the sweat duct is regulated by cAMP, consistent with the regulation of CF-affected Cl^- channels in secretory cells [1, 3, 6, 12, 19]. Preliminary evidence indicates that the G_{Cl} in the sweat duct has an anion selectivity sequence similar to CF-affected G_{Cl} in secretory cells [1, 4, 20] and only one type of Cl^- channel has been observed in both the apical and basolateral membranes of sweat ducts [15]. Thus, in cystic fibrosis the defect in G_{Cl} occurs in the basolateral as well as the apical membrane, which is an abnormality that probably extends to other absorptive tissues affected in this disease also. It is most likely that cAMP regulation is an intrinsic property of CF-affected Cl^- channels. This property is not selectively associated with the apical membrane only but may appear in either cell membrane as required by the specific transport function.

Conclusions

We conclude: (i) cAMP activation is a general property of CF-G_{Cl} present in absorption as well as in secretion, (ii) CF-G_{Cl} is expressed in both the apical and the basolateral membranes of sweat duct and is probably a property of other absorptive tissues affected in CF, and (iii) cAMP nonresponsiveness in some ducts is due to the prior, prolonged, endogenous activation of G_{Cl}.

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