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Down-Regulation of Cystic Fibrosis Transmembrane Conductance Regulator Gene Expression by Agents That Modulate Intracellular Divalent Cations

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In cystic fibrosis (CF), epithelial cells are unable to normally up-regulate apical membrane Cl^- secretion in response to agents which increase cyclic AMP, but they do increase Cl^- secretion in response to increases in intracellular Ca^{2+}. Since intracellular divalent cations regulate the expression of many genes, we hypothesized that mobilization of intracellular Ca^{2+} and/or other divalent cations might modulate not only Ca^{2+}-dependent Cl^- channels but also cystic fibrosis transmembrane conductance regulator (CFTR) gene expression. To evaluate this concept, HT-29 human colon carcinoma cells were cultured under various conditions designed to manipulate intracellular divalent cation concentrations and CFTR gene expression was quantified at the levels of transcription, mRNA accumulation, mRNA half-life, and protein. Exposure to the divalent cation ionophores A23187 and ionomycin (agents which increase intracellular divalent cation concentrations) caused dose- and time-dependent reductions of CFTR mRNA levels, which could be blocked by the use of Ca^{2+}- and Mg^{2+}-free media. Ionophore-induced CFTR gene modulation was also observed with T84 human colon carcinoma cells and freshly isolated normal human bronchial epithelial cells. Incubation of HT-29 cells with thapsigargin, an agent that releases Ca^{2+} from intracellular stores, or in medium containing increased extracellular concentrations of Ca^{2+} or Mg^{2+} also caused down-regulation of CFTR mRNA levels. Transcription run-on analysis showed that, parallel with the decrease in CFTR mRNA levels, A23187 reduced the rate of transcription of the CFTR gene, while CFTR mRNA transcript half-life was unaffected. Consistent with the down-regulation of CFTR gene expression, CFTR protein levels also decreased after exposure to A23187. Thus, despite the independence of Ca^{2+}-dependent Cl^- channels and cyclic AMP-dependent CFTR-related Cl^- channels in epithelial cells, increases in intracellular divalent cation concentrations down-regulate the expression of the CFTR gene at the transcriptional level, with consequent decreases in CFTR mRNA and protein.

Cystic fibrosis (CF), one of the most common fatal hereditary disorders in Caucasians, is caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, a 250-kb, 27-exon gene on chromosome 7 (7, 20, 28, 41). The translation product of CFTR mRNA is a 1,480-residue polypeptide with a predicted molecular mass of 168 kDa (28). The CFTR protein is glycosylated and can be phosphorylated by protein kinases (8, 10, 15, 30). The available evidence suggests that CFTR is a Cl^- channel on the apical surface of epithelial cells that responds to increased levels of intracellular cyclic AMP (cAMP) by secreting Cl^- from the cells (1, 2, 4, 19).

While the devastating consequences of mutations of the CFTR gene suggest that CFTR-mediated Cl^- secretion plays an important physiologic role, epithelial cells of the respiratory, pancreatic, and gastrointestinal tracts have other means of secreting Cl^- (3, 12, 34, 36). In this regard, whole-cell current studies of these epithelial cells show distinct responses to agents that elevate cAMP compared to agents that mobilize intracellular divalent cations (3, 12, 34, 36). Furthermore, when the normal CFTR cDNA is transferred into Sf9 insect cells, a cell type that does not normally secrete Cl^- in response to either agents that elevate cAMP or agents that mobilize intracellular divalent cations, the cells secrete Cl^- in response to agents that elevate intracellular cAMP but not to agents that mobilize divalent cations, strongly implying that the CFTR protein responds to the former and not to the latter (19). While the cAMP-dependent Cl^- secretory pathway involves protein kinase A, the Ca^{2+}-dependent pathway is mediated by stimulation of the multifunctional calcium/calmodulin-dependent protein kinase (3, 8, 12, 18, 23, 24, 32, 36, 37).

On the basis of these observations, the current working hypothesis is that the divalent cation-dependent Cl^- channel activity in these epithelial cells is distinct from CFTR. However, while this is likely to be the case at the protein level in terms of the function of these channels, it is possible that the cAMP-dependent and divalent cation-dependent pathways that modulate these distinct Cl^- channel activities are linked at the level of gene expression. To evaluate this concept, the present study was designed to examine the effect of increasing intracellular divalent cation concentrations on the expression of the CFTR gene. Using the HT-29 human colon carcinoma cell line as a model, we have observed a surprisingly strong link between divalent cation mobilization and the regulation of CFTR gene expression.

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MATERIALS AND METHODS

Cell culture and modulation of divalent cations. HT-29 colon adenocarcinoma cells (American Type Culture Collection; HTB 38), a cell line known to express the CFTR gene (25, 39, 40), were maintained in Dulbecco modified Eagle medium (Whittaker Bioproducts). Dulbecco modified Eagle medium includes 1.8 mM Ca^{2+} and 0.8 mM Mg^{2+} supplemented with 2 mM glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 10% fetal bovine serum. The cells were evaluated at confluency. T84 cells (28) (American Type Culture Collection; CCL 248) were cultured in the same way that HT-29 cells were, except with 5% fetal bovine serum. Normal human bronchial epithelium was obtained from mainstem or lobar bronchi by fiber optic bronchoscopy using a 1-mm cytology brush (33). The samples were immediately suspended in RPMI 1640 (Whittaker Bioproducts), collected by centrifugation, and cultured in LHC-8 medium (Biofluids). LHC-8 contains 0.11 mM Ca^{2+} and no Mg^{2+}) supplemented with 100 U of penicillin and 100 μg of streptomycin per ml in plates coated with 10 μg of human fibronectin (Calbiochem), 30 μg of collagen (Collagen Corporation), and 10 μg of bovine serum albumin (Sigma) per ml (21).

In experiments designed to increase intracellular divalent cation concentrations, duplicate cultures of cells were incubated with medium containing 0 to 5 μM A23187, 0 to 10 μM ionomycin (both from Calbiochem), or 0 to 500 nM thapsigargin (Sigma) (5, 31). In some experiments, divalent cations were eliminated from the medium by incubation in DMEM without Ca^{2+} and Mg^{2+} (NIH media unit) supplemented with 0.5 mM ethylenediaminetetraacetate (EDTA). In other studies, the extracellular divalent cation concentration was increased by incubation of the cells in Ca^{2+}- and Mg^{2+}-free medium supplemented with $CaCl_2$ and $MgCl_2$ at concentrations from 0 to 100 mM. Under the conditions used, cell viability, as measured by trypan blue exclusion, was ≥95% in all experiments. To verify that intracellular Ca^{2+} concentration increased as expected, the cellular uptake of $^{45}Ca^{2+}$ was quantified before and after treatment with A23187 and in high extracellular Mg^{2+} (50 mM). HT-29 cells were incubated in Dulbecco modified Eagle medium supplemented with $^{45}Ca^{2+}$ (0.5 μCi/ml; Amersham) and with or without A23187 for different times. The plates were then washed with phosphate-buffered saline (4°C) containing 10 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), and cells were harvested. Aliquots were taken for determination of total protein, and the radioactivity of the cell suspension was determined by scintillation counting. The increase of intracellular Ca^{2+} was quantified as the ratio of uptake per milligram of protein of resting cells to that of treated cells.

CFTR mRNA transcript levels. CFTR mRNA transcript levels in the cells were evaluated under various conditions by isolating total RNA by the guanidine thiocyanate-CsCl gradient method (11). Total RNA (10 μg per lane) was evaluated by Northern or slot blot hybridization by using a ^{32}P-labeled 4.5-kb CFTR cDNA probe (pTG4976), and mRNA levels were quantitated by densitometric scanning of the autoradiograms as previously described (32). As a control, γ-actin mRNA levels were evaluated in parallel by using a ^{32}P-labeled γ-actin cDNA probe (pHFγA-1) (16).

CFTR gene transcription rate. The rate of transcription of the CFTR gene was evaluated by nuclear transcription run-on analysis as previously described (39, 40). The DNA targets used were a 4.5-kb human CFTR cDNA (pTG4964), a human genomic c-*fos* clone, a human genomic c-*myc* clone, and the human β-actin cDNA pHFβA-1. The plasmid pUC19, containing no human DNA, was used as a negative hybridization control, and the 4.1-kb 28S mouse rRNA gene was used as a positive control. The transcription rate of cells treated with A23187 was expressed relative to the transcription rate of resting cells (defined as 100%).

CFTR mRNA stability. To estimate the stability of CFTR transcripts in resting cells and during periods of intracellular Ca^{2+} modulation, confluent HT-29 cells were incubated with or without 2 μM A23187 for 12 h. Actinomycin D (5 μg/ml; Calbiochem) was added, and incubation was continued for up to 24 h. RNA was extracted, the amount of CFTR mRNA was quantified as described above, and the CFTR mRNA transcript half-life was calculated (35).

CFTR protein levels. Relative amounts of CFTR protein in confluent HT-29 cells exposed to 2 μM A23187 for 24 or 48 h were evaluated by immunoprecipitation followed by phosphorylation using protein kinase and [γ-^{32}P]ATP, similar to that described by Cheng et al. (9). Briefly, cells were lysed in buffer (150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM Tris-HCl, pH 8.0) containing 1.5 μM 4-aminobenzoic acid and 7.5 μg of pepstatin A per ml (both from Fluka) plus 15 μg of chymostatin, 100 μg of aprotinin, and 50 μg of leupeptin per ml and 0.7 μM phenylmethylsulfonyl fluoride (all from Boehringer). DNA was sheared, and nonsolubilized material was removed by centrifugation. Equal amounts of total protein (measured with a Pierce protein assay reagent kit) were immunoprecipitated by the addition of 5 μl of a mouse monoclonal antibody raised against a CFTR exon 13-β-galactosidase fusion protein (Genzyme) and incubated at 4°C for 2 h. *Staphylococcus aureus* cells (Pansorbin, Calbiochem) precoated with rabbit anti-mouse immunoglobulin G (Cappel) were added and incubated at 4°C for an additional 1 h. Material bound to the *S. aureus* cells was washed three times with buffer (4°C) and then once with 150 mM NaCl–10 mM Tris-HCl, pH 7.5 (4°C). Immunoprecipitated proteins were phosphorylated with 10 μCi of [γ-^{32}P]ATP (NEN) plus 100 ng of the catalytic subunit of cAMP-dependent protein kinase (Sigma) in a final volume of 50 μl of kinase buffer (50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 100 μg of bovine serum albumin per ml) and incubated at 30°C for 1 h. Samples were electrophoresed on a 6% sodium dodecyl sulfate–polyacrylamide gel after incubation at 37°C for 10 min. Gels were dried under a vacuum, and autoradiograms were prepared.

RESULTS

Modulation of CFTR mRNA levels. Northern (RNA blot) analysis readily demonstrated the presence of the expected 6.5-kb CFTR mRNA transcripts in resting HT-29 cells (Fig. 1, lane 1). Interestingly, exposure to 2 μM A23187 for 12 h caused a striking reduction in the amount of CFTR transcripts (lane 2). As a control, γ-actin mRNA levels were unchanged under the same conditions (lanes 7 and 8).

Evaluation of T84 colon carcinoma cells and freshly isolated normal human bronchial cells demonstrated that the A23187-induced down-regulation of CFTR mRNA transcripts was not a process limited to HT-29 cells. In this regard, 6.5-kb CFTR transcripts were observed in both cell types (Fig. 1, lanes 3 and 5) but were reduced significantly in the presence of A23187 (lanes 4 and 6). In contrast, as with the HT-29 cells, γ-actin transcript levels in T84 and human

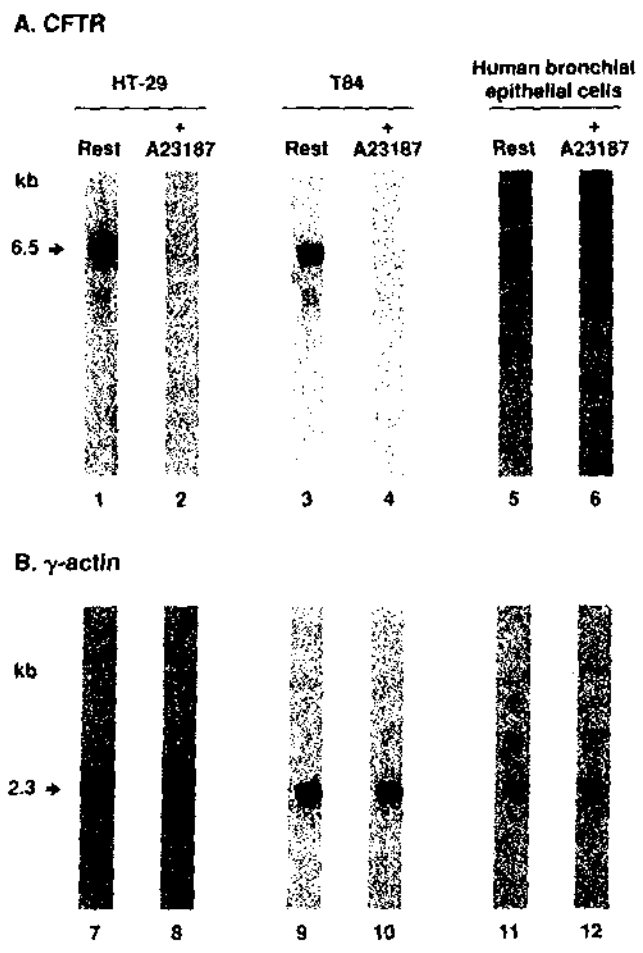


FIG. 1. Northern analysis demonstrating A23187-induced down-regulation of CFTR gene expression in HT-29 colon carcinoma cells, T84 colon carcinoma cells, and freshly isolated normal human bronchial epithelial cells. Lanes: 1, resting HT-29 cells (expressing 6.5-kb CFTR mRNA transcripts); 2, same as lane 1 but after 12 h of exposure to 2 μM A23187; 3 and 4, similar to lanes 1 and 2, respectively, but with T84 cells; 5 and 6, similar to lanes 1 and 2, respectively, but with bronchial epithelial cells; 7 to 12, similar to lanes 1 to 6, respectively, but evaluated with a γ-actin probe (2.3-kb transcripts).

bronchial cells were unchanged when they were exposed to A23187 (lanes 9 to 12).

Quantification of CFTR mRNA levels in HT-29 cells demonstrated that the effect of A23187 on CFTR mRNA levels was dose dependent. After 12 h of exposure to 2 μM A23187, CFTR mRNA levels had fallen to 13% of control values (Fig. 2A). The effect of A23187 on down-regulation of CFTR mRNA levels was also time dependent, reaching a nadir after 12 h of exposure to 2 μM A23187 and then returning slowly to resting levels (Fig. 2B). To demonstrate that this effect was attributable to modulation of intracellular divalent cation concentration and not to a nonspecific effect of A23187, ionomycin (another ionophore) was used in parallel experiments. Ionomycin caused a similar dose- and time-dependent reduction in CFTR mRNA levels (not shown).

To ensure that exposure to A23187 increased intracellular Ca^{2+} levels, $^{45}Ca^{2+}$ uptake was evaluated in resting and

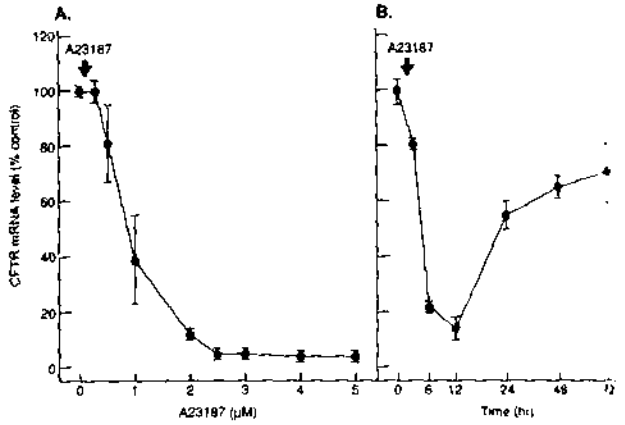


FIG. 2. Evaluation of dose- and time-dependent down-regulation of CFTR mRNA transcript levels in HT-29 cells by A23187. (A) Dose-dependent reduction of CFTR mRNA transcript levels. The dose-response effect of A23187 on CFTR mRNA transcript levels was evaluated by slot-blot hybridization using a 12-h incubation and A23187 concentrations from 0 to 5 μM. (B) Time-dependent reduction of CFTR mRNA transcript levels. Cells were incubated with 2 μM A23187 for 0 to 72 h. For panels A and B, each datum point is the average of duplicate determinations in two separate experiments.

A23187-treated HT-29 cells. Following administration of A23187, a peak of $^{45}Ca^{2+}$ accumulation that was 16-fold higher than that of resting cells was reached after 15 min of exposure. Over the next 48 h, the intracellular $^{45}Ca^{2+}$ levels remained elevated by about sevenfold over those of resting levels (data not shown). Increasing extracellular Mg^{2+} to 50 mM did not change the $^{45}Ca^{2+}$ uptake in HT-29 cells, suggesting that the observed decrease in CFTR mRNA transcript levels by higher concentrations of extracellular Mg^{2+} was not due to an elevation of intracellular Ca^{2+} levels, but was a general effect of divalent cations (not shown).

To show that Ca^{2+} and/or Mg^{2+} influx into the cell was responsible for the down-regulation of CFTR mRNA levels by A23187, experiments with A23187 were performed in Ca^{2+}- and Mg^{2+}-free medium supplemented with 0.5 mM EDTA. Incubation of the cells in Ca^{2+}- and Mg^{2+}-free medium completely blocked the down-regulation of CFTR mRNA levels by A23187 (not shown). To further evaluate the effect of divalent cations, the concentrations of Ca^{2+} and Mg^{2+} were increased in the extracellular milieu by up to 100 mM. Increasing the extracellular levels of both cations caused a dose-dependent down-regulation of CFTR mRNA levels (Fig. 3). Thapsigargin, an agent that releases Ca^{2+} from intracellular stores, also down-regulated CFTR mRNA levels in HT-29 cells in a dose-dependent fashion after incubation for 12 h (Fig. 4).

Evaluation of CFTR gene transcription rate and CFTR mRNA half-life. Consistent with the A23187-induced reduction of CFTR mRNA transcript levels, addition of A23187 to HT-29 cells caused a suppression of the rate of transcription of the CFTR gene (Fig. 5). As previously noted (39), the CFTR gene is transcribed at a low rate in resting HT-29 cells. In the presence of A23187, the transcription rate was down-regulated even more, to approximately 50% of the rate of CFTR transcription in resting cells. As for the controls, the transcription rates of *c-fos*, *c-myc*, and rRNA genes were unchanged by exposure to A23187.

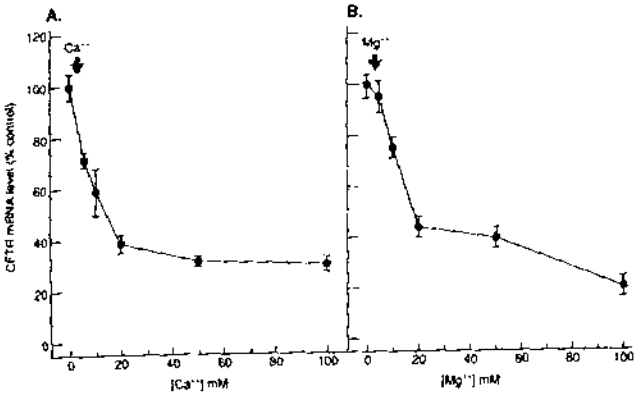


FIG. 3. Evaluation of dose-dependent down-regulation of CFTR mRNA transcript levels in HT-29 cells by increasing extracellular Ca^{2+} and Mg^{2+} concentrations. (A) Increasing extracellular Ca^{2+} concentration. Cells were incubated in medium supplemented with 0 to 100 mM Ca^{2+} for 12 h; the Mg^{2+} concentration was kept constant at 0.8 mM. (B) Increasing extracellular Mg^{2+} concentration. Cells were incubated in medium supplemented with 0 to 100 mM Mg^{2+} for 12 h; the Ca^{2+} concentration was kept constant at 1.8 mM. For panels A and B, the data were determined by slot-blot hybridization. Each datum point is the average of duplicate determinations in two separate experiments.

To examine whether the reduction in CFTR mRNA levels by A23187 was associated with a change in CFTR mRNA transcript stability, the half-life of CFTR mRNA was evaluated in A23187-treated HT-29 cells and compared with that in resting cells. In resting cells, CFTR transcripts had a half-life of about 13.0 ± 0.6 h (mean ± standard error of the mean) (Fig. 6). This value was similar to that observed for A23187-treated cells (13.1 ± 1.2 h).

Modulation of CFTR protein levels. Consistent with the observations that HT-29 cells actively transcribe the CFTR gene and contain CFTR mRNA transcripts, the CFTR protein was present in cell lysates, with a major band of 190 kDa. Consistent with the A23187-induced down-regulation

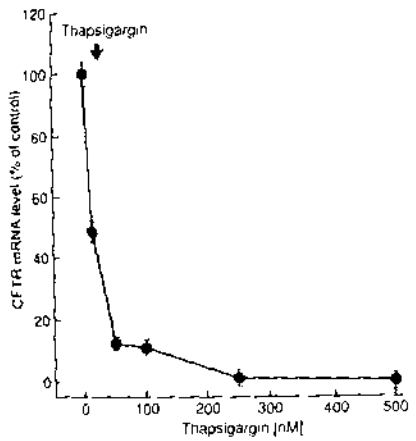


FIG. 4. Dose-dependent down-regulation of CFTR mRNA transcript levels in HT-29 cells by increasing concentrations of thapsigargin. The cells were incubated for 12 h in the presence of 0 to 500 nM of thapsigargin. Data were obtained by slot-blot hybridization. Each datum point is the average of duplicate determinations in two separate experiments.

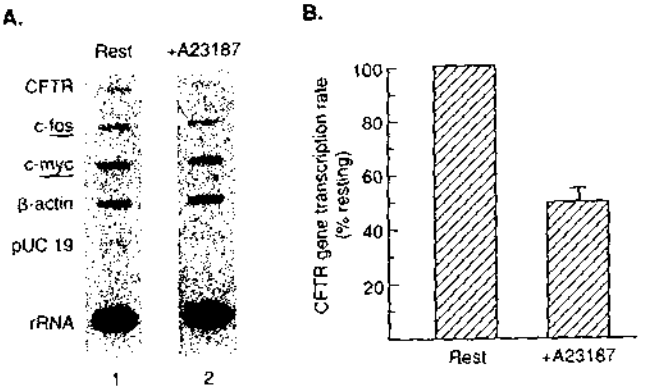


FIG. 5. A23187-induced down-regulation of the relative rate of CFTR gene transcription. CFTR gene transcription rate was evaluated by nuclear run-on analysis in comparison with those of *c-fos*, *c-myc*, β-actin, pUC19 (an irrelevant plasmid), and rRNA. (A) Lanes: 1, resting HT-29 cells; 2, HT-29 cells after exposure to 2 μM A23187 for 12 h. (B) Comparison of the relative transcription rate of the CFTR gene in resting and A23187-treated cells. Data are averages of four separate experiments.

of transcription of CFTR gene and the associated reduction of CFTR mRNA levels, after 12 h of exposure to 2 μM A23187, CFTR protein levels were substantially reduced (Fig. 7).

DISCUSSION

There is increasing evidence that epithelial cells that utilize the CFTR/cAMP-related Cl^- secretory pathway also have a Ca^{2+}-dependent mechanism to augment Cl^- secretion (3, 8, 12, 34, 36). Data from several sources suggest that these pathways are physiologically separate and that they probably involve different Cl^- channels (3, 4, 8, 12, 19, 34, 36). The Ca^{2+}-dependent channel has not yet been identi-

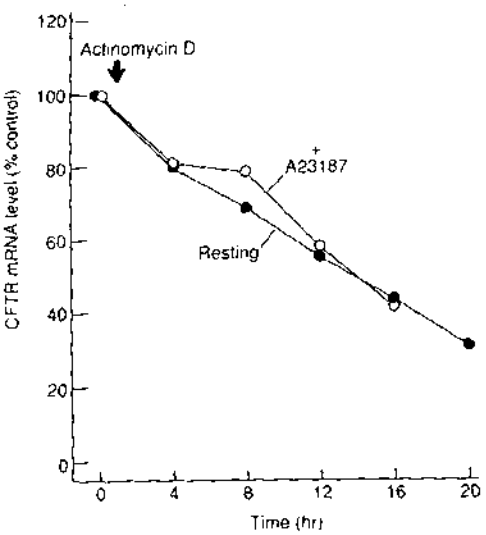


FIG. 6. Evaluation of the stability of CFTR mRNA transcripts in HT-29 cells before and after exposure to A23187. After 12 h of exposure to 2 μM A23187, RNA synthesis was inhibited by addition of actinomycin D, and CFTR mRNA levels were evaluated by slot-blot hybridization at various time points. Data are averages of three separate experiments.

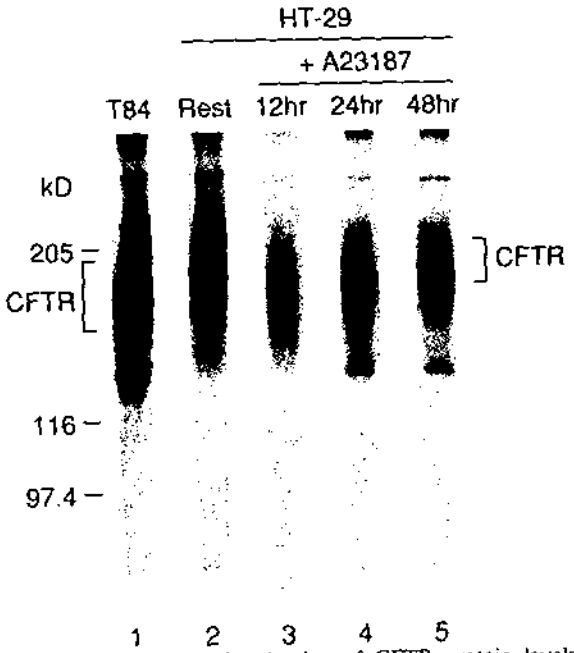


FIG. 7. A23128-induced reduction of CFTR protein levels in HT-29 cells. Lysates of the cells were evaluated by sodium dodecyl sulfate-polyacrylamide gels after immunoprecipitation with an anti-CFTR antibody and exposure to kinase in the presence of [γ-^{32}P]ATP. Lanes: 1, resting T84 cells; 2, resting HT-29 cells; 3, HT-29 cells after 12-h exposure to 2 μM A23187; 4, same as lane 2, but after 24-h exposure to A23187; 5, same as lane 2, but after 48-h exposure to A23187. The CFTR protein is indicated; CFTR in HT-29 cells migrates with a higher molecular mass than CFTR in T84 cells. A23187 causes a marked decrease in CFTR levels by 12 h. The pattern shown is a representative of four separate experiments.

fied, but recent evidence suggests that the CFTR gene product is the cAMP-dependent Cl^- channel (1, 2, 4, 19). The present study demonstrates that despite their functional independence at the protein level, these pathways are linked at the level of gene expression. In this regard, signals that raise intracellular divalent cation levels also down-regulate expression of the CFTR gene in epithelial cells.

Regulation of CFTR gene expression. While Cl^- secretion in epithelial cells through the Ca^{2+}-dependent channel and the CFTR/cAMP-dependent channel represent independent processes and distinct channels, their interrelationship at the level of gene expression has interesting and potentially important implications. It is intriguing that by increasing intracellular Ca^{2+} and Mg^{2+} concentrations, the epithelial cell activates Cl^- secretion by one pathway yet turns off the expression of a gene for Cl^- secretion by another pathway. This suggests possible feedback from the physiologic level to the gene level. It is not clear what constitutes this mechanism, but the regulation appears to be at the level of transcription of the CFTR gene. However, because cellular levels of CFTR mRNA were reduced to 13% after 12 h of A23187 exposure while the rate of transcription of the CFTR gene was decreased to only 50% of that of resting cells with no change in CFTR mRNA half-life, it is conceivable that there are other regulatory mechanisms involved in the Ca^{2+}-mediated down-regulation of CFTR gene expression, possibly related to intracellular degradation of nascent RNA or the rate of transportation of mRNA from the nucleus to the cytoplasm. Alternatively, the difference between the level of reduction of CFTR gene transcription rate and CFTR mRNA levels could be technical artifacts arising from differences in the handling of the cells for the two assays.

Several lines of evidence suggest that the observed down-regulation of CFTR gene expression in epithelial cells was mediated through a pathway related to a mobilization of intracellular divalent cations. First, the uptake of $^{45}Ca^{2+}$ in HT-29 cells was clearly increased after A23187 exposure. Second, the observations obtained with A23187 (a divalent cation ionophore with a selectivity of $Mn^{2+} > Ca^{2+} = Mg^{2+} > Sr^{2+} > Ba^{2+}$ [5]) were paralleled by those obtained with another divalent cation ionophore, ionomycin (with an ion selectivity of $Ca^{2+} > Mg^{2+} \gg Sr^{2+} = Ba^{2+}$ [5]), suggesting that the down-regulation by A23187 is a property related to its ability to cause an influx of extracellular divalent cations. Third, thapsigargin, an agent that increases intracellular Ca^{2+} by a nonionophoric mechanism causing release from intracellular stores (31), also down-regulates CFTR mRNA levels. Fourth, an increase of Ca^{2+} or Mg^{2+} concentration in the medium resulted in concentration-dependent down-regulation of CFTR mRNA levels. Finally, depletion of Ca^{2+} and Mg^{2+} from the extracellular medium eliminated the down-regulation of CFTR gene expression by A23187. Importantly, this observed down-regulation was specific for CFTR, since mRNA levels for γ-actin, a structural cytoplasmic protein, did not change after intracellular Ca^{2+} and Mg^{2+} were increased.

It is known that differentiation of HT-29 cells up-regulates CFTR mRNA levels (25), and it is therefore conceivable that ionophores might dedifferentiate these cells and so cause the observed down-regulation of CFTR gene expression. This is not likely in our studies, because (i) cells do not dedifferentiate in hours, in which the effect of the ionophores on CFTR gene expression was observed, and (ii) an increase of intracellular Ca^{2+} usually leads to differentiation of cells and not to dedifferentiation (6). The divalent cation-mediated down-regulation of CFTR in HT-29 cells was not specific for the HT-29 cell line, as the T84 colon carcinoma cell line and freshly isolated human bronchial cells showed a decrease of CFTR mRNA levels after A23187 treatment in a fashion analogous to that of HT-29 cells. The latter observation is particularly relevant to CFTR, since the major manifestations of CF are on the epithelial surfaces of the airways and airway epithelial cells have both cAMP- (i.e., CFTR) and divalent cation-dependent Cl^- channels (3, 7, 8, 18, 23, 24, 27, 34, 36, 38).

Structural characterization of 3.8 kb of genomic DNA 5′ to exon I of the CFTR gene demonstrates features of a housekeeping gene promoter, such as no TATA box but sequences with homology to a CCAAT box and GC boxes, a high GC content, and multiple transcription start sites (39). Despite this, the CFTR promoter also has elements suggesting that it is regulatable, including DNA sequences homologous to a glucocorticoid response element, a cAMP response element, and an AP-1-binding site (39). Consistent with this concept, CFTR down-regulation by increased intracellular cations appears to be modulated at the transcriptional level. This may be similar to the down-regulation of CFTR gene expression in the human colon carcinoma cell line T84 by phorbol myristate acetate, an agent capable of activating AP-1 elements (22, 32, 39). Relevant to the observations in the present study, phorbol myristate acetate can also activate protein kinase C in many cell types, including T84 cells, as can agents that mobilize divalent cations (26). Therefore, the down-regulation of expression of the CFTR gene by

agents that increase intracellular divalent cations may be working through a pathway involving protein kinases.

Implications of the link between Ca^{2+}- and CFTR/cAMP-mediated Cl^- secretion for the therapy of CF. As described recently (34), the Ca^{2+}-dependent Cl^- secretory pathway in epithelial cells involves a calcium/calmodulin-dependent protein kinase. This pathway, in contrast to the cAMP-mediated Cl^- secretion, is fully functional in CF, an observation with potential implications for therapy for CF, i.e., the Ca^{2+}-dependent Cl^- secretory pathway might be a way to circumvent the CF defect because CF airway cells retain calcium/calmodulin-dependent protein kinase regulation of Cl^- current. Furthermore, there is evidence suggesting that the complete absence of expression of CFTR is associated with less severe respiratory disease than is the common ΔF508 mutation in CFTR (13, 14, 17). Thus, down-regulation of the CFTR gene might be beneficial for most individuals with CF. In this context, agents that specifically activate the Ca^{2+}-dependent Cl^- secretory pathway or stimulate phosphorylation of the Cl^- channel regulator by calcium/calmodulin-dependent protein kinase may be able to circumvent the CF defect by using the alternate Ca^{2+}-dependent pathway and to down-regulate expression of the mutant gene at the transcriptional level and thus at the mRNA and protein levels.

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