Aspirin and some other nonsteroidal anti-inflammatory drugs inhibit cystic fibrosis transmembrane conductance regulator protein gene expression in T-84 cells

CYSTIC fibrosis (CF) is caused by mutations in the CF gene, which encodes CF transmembrane conductance regulator protein (CFTR), a transmembrane protein that acts as a cAMP-regulated chloride channel. The disease is characterized by inflammation but the relationship between inflammation, abnormal transepithelial ion transport, and the clinical manifestations of CF are uncertain. The present study was undertaken to determine whether three nonsteroidal anti-inflammatory drugs (NSAIDs) (aspirin, ibuprofen, and indomethacin) modulate CFTR gene expression in T-84 cells. Treatment with NSAIDs reduced CFTR transcripts, and decreased cAMP-stimulated anion fluxes, an index of CFTR function. However, the two phenomena occurred at different concentrations of both drugs. The results indicate that NSAIDs can regulate both CFTR gene expression and the function of CFTR-related chloride transport, and suggest that NSAIDs act via multiple transduction pathways.

Keywords: CFTR, Gene expression, T-84 Cells, Aspirin, Nonsteroidal anti-inflammatory drugs

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Introduction

Inflammation in response to an external attack or internal malfunction triggers the synthesis of many extra- and intracellular mediators that can modulate the activity of the cell's membrane, cytoplasm and nucleus. The inhibitory effects of aspirin on most inflammatory responses have long been attributed to its ability to block the synthesis of prostaglandins. These natural autacoïds produced by cyclooxygenase activation provoke inflammation.[1] It is now well established that aspirin and the other nonsteroidal anti-inflammatory drugs (NSAIDs) also modulate membrane[2–6] and nuclear[7–9] responses independently of the inhibition of cyclooxygenase. This raises questions about the molecular reactions contributing to the beneficial effects of NSAIDs on the symptoms of cystic fibrosis (CF).[10]

CF transmembrane conductance regulator protein (CFTR) is known to act as a cAMP-activated anionic channel. Clinical manifestations of CF are correlated with mutations in the CF gene, which cause the protein it makes to function abnormally.[11] However, CF involves not only hydroelectrolytic abnormalities,

but also chronic inflammation and infection, and anti-inflammatory therapies improve the clinical condition of patients. The link between changes in anionic transmembrane transport and inflammation is uncertain, and some observations suggest that NSAIDs may have a dual effect in CF, combining inhibition of inflammation with the modulation of anion transmembrane transport.

Several effects of rapid modulations in ionic conductances by NSAIDs have been described. For example, the fenamates (niflumic and flufenamic acids) block the nonselective ion channels in rat exocrine pancreas,[3] or activate the voltage-dependent K current expressed in Xenopus oocytes.[6] Several reports indicate that NSAIDs alter chloride conductances in epithelial cells. Short applications of NSAIDs inhibit Ca^{2+}- and cAMP-regulated chloride secretion of cultured tracheal epithelial cells.[4] The fenamates also modulate chloride conductance in bovine retinal pigment epithelium, but in a more complex way.[5] Recently, it was reported that ibuprofen blocks the CFTR-mediated chloride secretion in T-84 cells and in human and mouse tracheal epithelia.[2] Other data suggest that aspirin and other NSAIDs

might modulate CFTR gene expression by modulating various transcription factors which bind to specific nucleotidic sequences present in the CFTR promoter.[12] Aspirin has been shown to inhibit the activation of transcription factors such as NFκB[7] and AP$_1$[8], and to facilitate the binding capacity of the Heat Shock Factor, HSF1.[9] Indomethacin has also been shown to inhibit the activation of AP-1.[8] We suggested that aspirin may control CFTR gene expression. This would not be the first 'nuclear effect' of the drug, since aspirin had already been shown to suppress inflammation-induced expression of interleukin-1 (Il-1), Il-6 and adhesion molecules in Hela cells[7] and to inhibit the synthesis of IL-1-induced PGH synthase.[13]

The present study was undertaken to determine whether aspirin, ibuprofen, and indomethacin modify CFTR gene function. We did this by measuring CFTR transcripts and cAMP-stimulated anion fluxes, these being indices of CFTR function in T-84 cells.

Materials and methods

Cell culture and drug treatment

Human T-84 carcinoma cells were obtained from the American Type Culture Collection (Rockville, MD) and propagated in a mixture (1:1) of Dubelcco's modified Eagle's medium (DMEM) and Ham's F-12 medium containing 15 mM Hepes and 10% fetal calf serum (FCS). LLCPK cells stably transfected with wild type CFTR, ΔF_{508}-CFTR, and nontransfected cells were both cultured in DMEM medium.

The cells were seeded at a density of $2 \times 10^4/cm^2$. Stock solutions of aspirin (1 mol/l), indomethacin (5×10^{-3} mol/l), and ibuprofen (10^{-2} mol/l) were made up in ethanol. On the third day after passage, the cells were treated with aspirin or the other NSAIDs in FCS-enriched medium. RNA was analyzed after 24 h of treatment (except for kinetic studies). Functional studies were performed on T-84 cells incubated with the drugs for 48 h. Control cells received the same volume of solvent (<1%). All the culture materials were obtained from Life Technologies (Les Ulis, France); the drugs were from Sigma (France).

Analysis of cell viability

In a preliminary series of experiments we verified that, even at the highest concentrations used in this study, the drugs were not toxic. Cells maintained under control conditions, or after a 48-h treatment with aspirin (10^{-3} mol/l), indomethacin (2×10^{-5} mol/l), or ibuprofen (5×10^{-4} mol/l) were washed with phosphate-buffered saline (PBS), trypsinized, suspended in serum-free medium, and centrifuged. Cells were washed twice more with PBS and suspended in PBS. Parts of this suspension were used to test cell viability with trypan blue, for counting cells in a Malassez cell, and to measure protein concentration according to Lowry *et al.*[14]

Cell counting and trypan blue exclusion test showed that when 10^{-3} mol/l aspirin was added to T-84 cells for a 48-h incubation, it neither killed them nor altered their proliferation rate. The same treatment also had no effect on the protein content of the culture. The same results were observed after 48-h incubation with 2×10^{-5} mol/l indomethacin and 5×10^{-4} mol/l ibuprofen (Table 1).

RNA extraction and analysis

Total RNA was isolated with phenol/chloroform[15] using the Trizol reagent (Life Technologies), according to the manufacturer's instructions. The RNA was then fractionated on 0.9% agarose gels (15 μg/well), transferred to nylon membranes (Promega Charbonnières, France) and fixed by heating. The filters were hybridized to ^{32}P-labeled cDNA probes (specific activity >10^9 cpm/mg) with the Quik Hyb protocol provided by Stratagene (Ozyme, Les Ulis, France), washed under stringent conditions (0.1 SSC, 0.1% sodium docdecyl sulfate at 52°C for 20 min) and autoradiographed at −80°C. The CFTR probe was the 1.5-kb *Eco*R1-*Eco*R1 fragment of human CFTR-cDNA probe labeled by random priming. The membranes were rehybridized with a human β-actin cDNA probe from Oncogene Science (France Biochem, Meudon, France). The mRNAs were quantified by densitometric scanning of the autoradiograms on an ImageMaster

Table 1. Effects on NSAIDs on cell proliferation

	Number of cells (10^6 cells/well)	Living cells (% of total)	Cell protein (μg/well)
Control	1.55±0.11	90.9±2.4	220±25
Aspirin (5×10^{-4} mol/l)	1.85±0.25	91.3±2.7	234±21
Aspirin (10^{-3} mol/l)	1.40±0.06	91.4±3	223±30
Indomethacin (2×10^{-5} mol/l)	1.40±0.15	89.2±2.9	205±28
Ibuprofen (5×10^{-4} mol/l)	1.63±0.22	90.5±2.6	220±21

The number of cells per well, the percentage of living cells, and the protein content were determined as described in materials and methods on cultures maintained under control conditions or treated for 48 h with the various NSAIDs. Each value is the mean ±SE of three determinations performed in triplicate on three different cultures.

VSD (Pharmacia-Biotech-Amersham, Orsay, France), and CFTR mRNA amounts were normalized to those of β-actin. All experiments were repeated at least four times.

6-Methoxy-*N*-ethylquinolinium fluorescence assay

The 6-methoxy-*N*-ethylquinolinium (MEQ) was synthesized in the laboratory according to the method described by Biwersi and Verkman.[16] 6-Methoxyquinoline (20 mmol) and iondoethane (40 mmol) were allowed to react for 1 h with continuous stirring and boiling. After cooling, the reaction product was resuspended in ether, and filtered. The precipitate was washed twice in ether, then dissolved in ethanol/water (20:1) by mild heating, avoiding the boiling point. The undissolved residue was removed by filtration at 80°C through glass wool, and the solution was allowed to cristallyze on ice. The final product yielded 85% 6-methoxy-*N*-ethylquinolinium iodide: a bright yellow solid with a fusion point of 184°C. The properties of MEQ fluorescence were defined on a Stern–Volmer plot ($F_0/F = 1 + K_{Cl}[Cl]$, in which F_0 and F are the fluorescence intensities in the absence and presence of chloride, and [Cl] corresponds to the chloride

concentration. The data were obtained by measuring the fluorescence of KCl solutions (from 0 to 50 mM) supplemented with 1 μM MEQ (excitation, 350 nm; emission, 440 nm; filters were from Chroma (Paris, France)) using a SPEX Fluorolog spectrofluorometer and analyzed with DM3000 software (Jobin Yvon, France). The plot was linear, with a Stern–Volmer constant of 149 M^{-1} (Fig. 1). The plots obtained using the same solutions supplemented with the different NSAIDs (aspirin, indomethacin, ibuprofen) did not modify the Stern–Volmer constant (Fig. 1).

The measurements of intracellular MEQ fluorescence change were carried out as previously described.[17] Briefly, T-84 cells subcultured on glass slides (control cells, and those treated for 48 h with NSAIDs) were washed from culture medium, then loaded with MEQ and I^- for 10 min in hypotonic iodine solution (1:2 dilution of the isotonic solution, pH 7.4, adjusted with NaOH, containing the following reagents: 138 mmol/l NaI, 2.4 mmol/l K_2HPO_4, 10 mmol/l Hepes, 1 mmol/l $CaCl_2$, 10 mmol/l glucose) and allowed to recover for 10 min in isotonic I^- solution before being placed in a perfusion chamber on the stage of an inverted microscope (Diaphot, Nikon, France), where they were continuously perfused at 37°C with isotonic iodide solution. After a 2 min
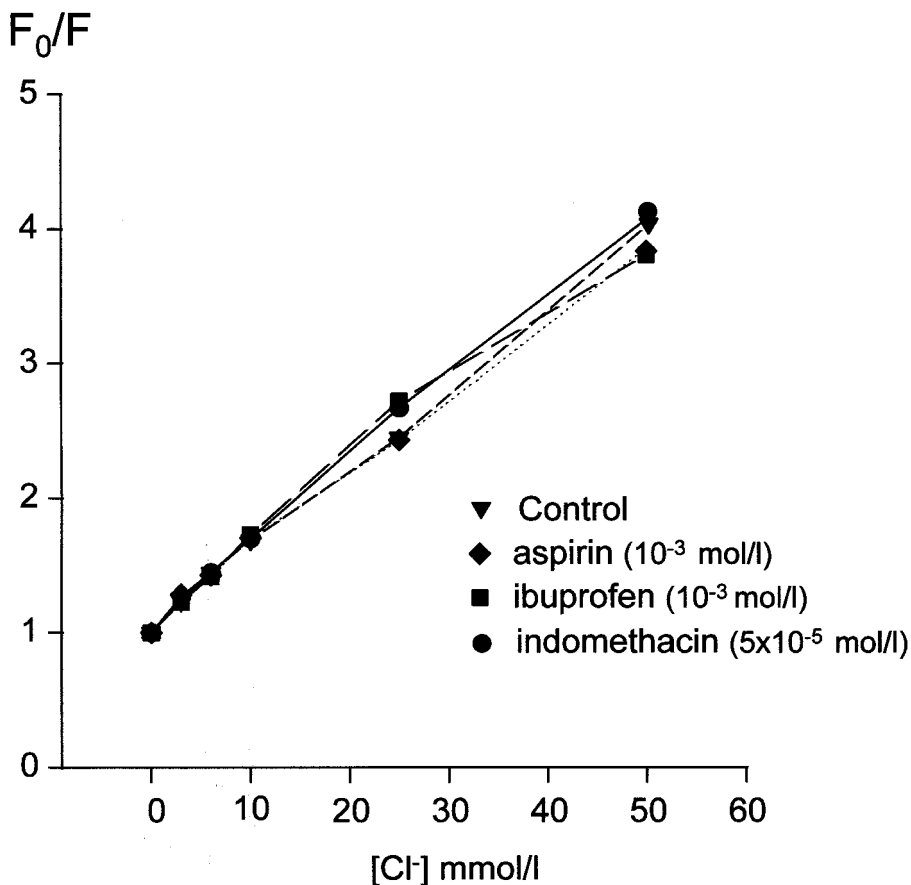

FIG. 1. Stern–Volmer plot. The plot was established by measuring the fluorescence of a 1 mmol/l MEQ solution, to which was added increasing amounts of KCl from 0 up to 50 mmol/l. The determinations were performed in water alone (▼), then with 10^{-3} mol/l aspirin (♦), 10^{-3} mol/l ibuprofen (■), or 5×10^{-5} mol/l indomethacin (●).
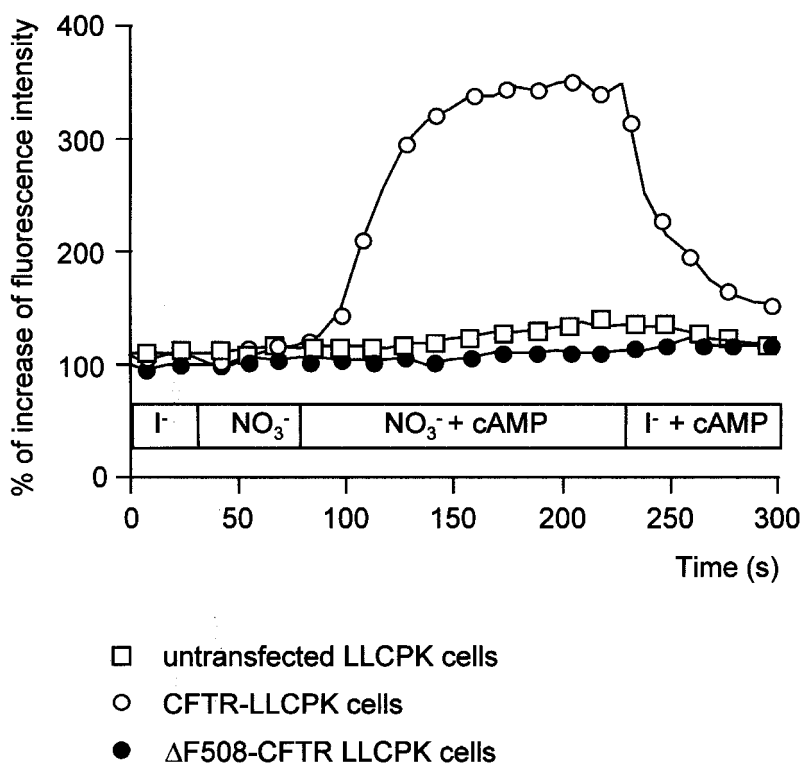
FIG. 2. Validation of the MEQ technology. The fluorescence of intracellular MEQ was registered in untransfected LLCPK cells (□) and in LLCPK cells expressing either the wild type CFTR (○) or the ΔF_{508}-CFTR (●), under sequential anionic substitutions of the superfusing medium and addition of permeant cpt-cAMP, as indicated in the inset and described in materials and methods.

perfusion period, I^- ions were replaced by NO_3^- ions. Because nitrate does not interact with MEQ, fluorescence increases as cell iodide flows from the cell through anion pathways in the plasma membrane, unmasking possible basal anion conductance. Changes in fluorescence under basal conditions, and with the cAMP cocktail (5×10^{-4} mol/l 8-(4-chlorophenylthio)-cAMP (cpt-cAMP) and 10^{-4} mol/l IBMX) in the nitrate perfusion solution were recorded. The whole experiment was performed without NSAIDs in the perfusion solutions. Fluorescence from the MEQ was measured in single cells with a digital imaging system and a CDD camera (Photonics Science, UK), and the results were analyzed using Imstar software (Paris, France). The initial rate of increase of MEQ fluorescence was measured in basal ($\Delta F_{basal}/\Delta t$) and stimulatory ($\Delta F_{cAMP}/\Delta t$) conditions.[17]

To validate the MEQ technology as a method for investigating CFTR gene function, we used LLCPK cells stably transfected with the wild-type or the $\Delta F508$-CFTR-mutated human cDNA CFTR gene.[18] The cells were then assayed with the MEQ technology already described. Figure 2 shows that the function of CFTR gene induces an increase in the rate of fluorescence after the addition to the perfusate of the cAMP cocktail in the CFTR-LLCPK cells. No cAMP-dependent fluorescence changes were detected in LLCPK nontransfected cells or $\Delta F508$-CFTR-LLCPK

cells. Thus, the MEQ assay can be taken as an index of CFTR activity.

Statistical analysis

Statistical analysis was by unpaired Student's *t*-test, and, where appropriate, by analysis of variance, with $P < 0.05$ considered statistically significant.

Results

Variation in CFTR mRNA levels

The modulation of CFTR gene expression by NSAIDs was investigated by Northern blot analysis, performed on proliferating cells.

The relative amount of CFTR gene products (normalized to β-actin products) was first determined in cells treated for 24 h with aspirin, ibuprofen and indomethacin. Figure 3A and B shows that, under our experimental conditions, aspirin (5×10^{-4} mol/l), ibuprofen (5×10^{-4} mol/l), and indomethacin (2×10^{-5} mol/l) reduced the amount of CFTR mRNA in T-84 cells.

After treating the cells for 24 h with aspirin, the amount of CFTR mRNA was unaltered by low concentrations of the drug, and was decreased by the concentrations larger than 10^{-4} mol/l (Fig. 4A). A half-
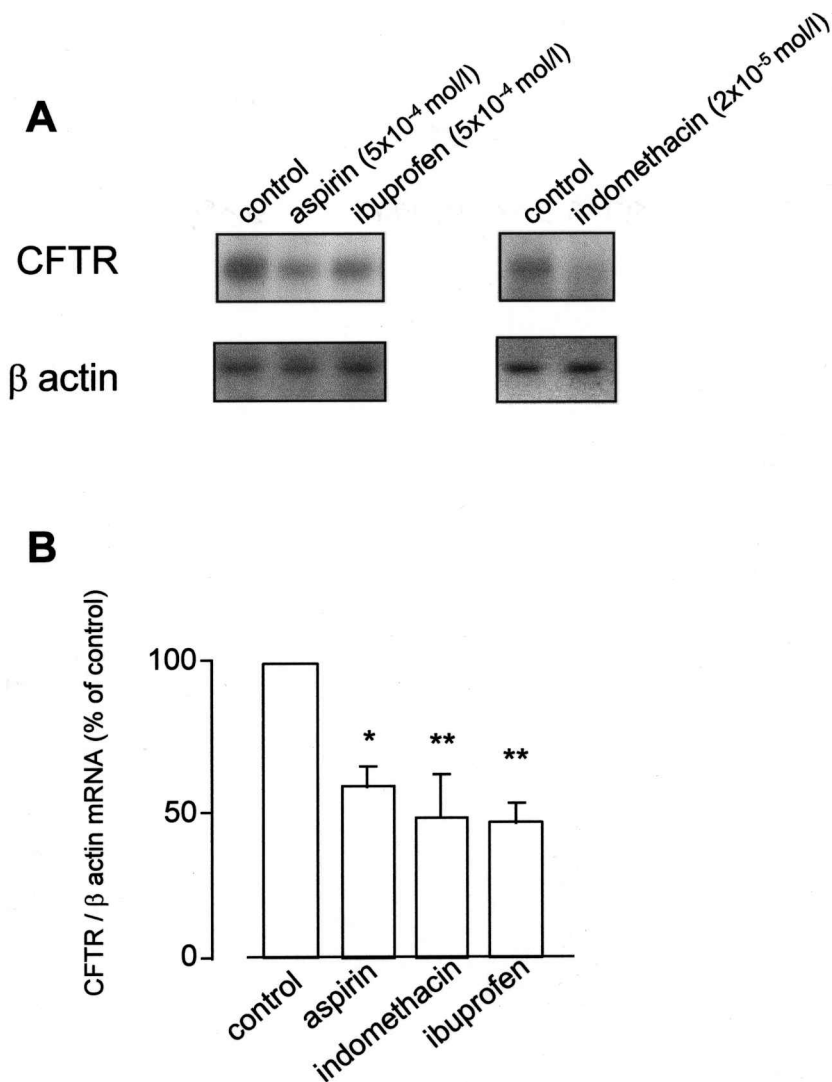
A

B

FIG. 3. Effects of aspirin, indomethacin, and ibuprofen on CFTR mRNA content in T-84 cells. (A) Representative Northern blot performed with 15 μg total RNA extracted from cells treated for 24 h with the indicated concentrations (mol/l) of NSAIDs. (B) Amounts of CFTR transcripts normalized to those of β-actin mRNA. Each value is the mean±SE of four experiments. * $P<0.05$; ** $P < 0.01$.

maximal inhibition was observed with aspirin concentrations close to 10^{-3} mol/l. β-actin transcripts were unchanged. Figure 4B shows the action of aspirin in relation to time, with the concentration 2×10^{-3} mol/l (corresponding to an 80% inhibition of CFTR transcripts after 24 h of treatment) chosen for these experiments. After 6 h of treatment, a 50% inhibition was observed, and the effect was greatest after 9 h. Together, the data show that the CFTR down-regulation is concentration and time dependent. Indomethacin (10^{-5} and 2×10^{-5} mol/l) and ibuprofen (10^{-4} and 5×10^{-4} mol/l) also significantly decreased CFTR mRNA levels in a concentration-dependent manner (Fig. 5). The cell content in β-actin mRNA was not altered by indomethacin or ibuprofen at any concentration (result not shown).

A relationship between the anti-inflammatory effects of aspirin, indomethacin, and ibuprofen, and their ability to decrease CFTR mRNA might proceed from their capacity to inhibit cyclooxygenase activity. To test this hypothesis, exogenous prostaglandin E_2 (PGE_2) was added to the cells at the same time as the NSAIDs. As shown in Fig. 6, 10^{-6} mol/l exogenous PGE_2 by itself decreased cell CFTR mRNA content, and when added with aspirin, indomethacin, and ibuprofen, it did not prevent their down-regulating effect. Therefore, the decrease in CFTR gene expression induced by the NSAIDs cannot be linked to the inhibition of cyclooxygenase activity.

Functional studies: MEQ assay

To determine whether the NSAIDs modulated the function of CFTR protein, we performed a MEQ assay, in the absence of the drugs, on T-84 cells treated for 24 and 48 h with 5×10^{-4} mol/l aspirin, 5×10^{-4} mol/l ibuprofen, and 2×10^{-5} mol/l indomethacin (Table 2). The results were compared with
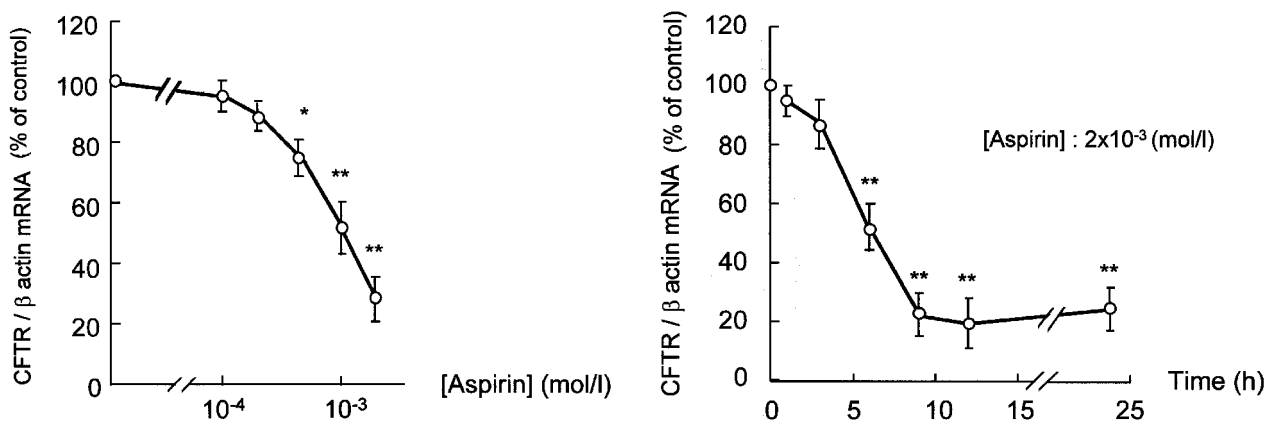
FIG. 4. Northern blot analysis of concentration and time dependence of CFTR mRNA decay induced by aspirin in T-84 cells. The Northern blots were performed using 15 μg total mRNA. The cell content in CFTR mRNA are normalized to the content in β-actin mRNA, and the results are expressed as a percentage of the ratio determined on control cells. (A) Concentration dependence. Cells were treated for 24 h with 0, 10^{-4}, $5×10^{-4}$, 10^{-3}, and $2×10^{-3}$ mol/l aspirin. Each value is the mean ±SE of six determinations. * $P<0.05$; ** $P<0.01$. (B) Time dependence. Cells were treated with $2×10^{-3}$ mol/l aspirin for 0, 1, 3, 6, 9, 12 and 24 h. Each value is the mean ±SE of five determinations. ** $P<0.01$.

those obtained with untreated cells studied on the same day. In the control (untreated) T-84 cells, addition of the permeant cAMP (cpt-cAMP, $5×10^{-4}$ mol/l) to the nitrate-containing solution augmented the rate of increase of MEQ fluorescence, quantified by the ratio $\Delta F_{cAMP}^{ctl}/\Delta F_{basal}^{ctl}$ (mean values, 7.8; $n=28$; Table 2). This points to activation of the cAMP-regulated anion pathway(s) (Fig. 7). The 24-h treatment of T-84 cells with either drug did not change the cpt-cAMP-induced increase in the rate of MEQ fluorescence observed in NO_3^--containing solutions (data not shown). On the other hand, the cpt-cAMP-induced increase in the rate of MEQ fluorescence observed in NO_3^--containing solutions was altered in cells treated during 48 h with the NSAIDs. It was abolished in the cells treated with aspirin

(10^{-3} mol/l), (Fig. 7A and Table 2, row 'aspirin', comparing columns 2 and 5, or columns 3 and 6) and decreased in the cells treated for 48 h with $5×10^{-4}$ mol/l ibuprofen (Fig. 7B and Table 2, row 'ibuprofen', comparing columns 2 and 5, or 3 and 6) or $2×10^{-5}$ mol/l indomethacin (Table 2, row 'indomethacin', comparing column 2 and 5, or 3 and 6).

Using the MEQ methodology, we have thus shown a decrease in cAMP-induced anionic flux provoked by the treatment of the cells with aspirin, indomethacin, and ibuprofen, and also demonstrated a particular action of ibuprofen and aspirin on T-84 cell basal anion conductance. Under our experimental protocol, this basal anion conductance (evidenced by the light emission provoked by replacing I^- by NO_3^- in the superfusing solution) was small (as Fig. 7A and Table
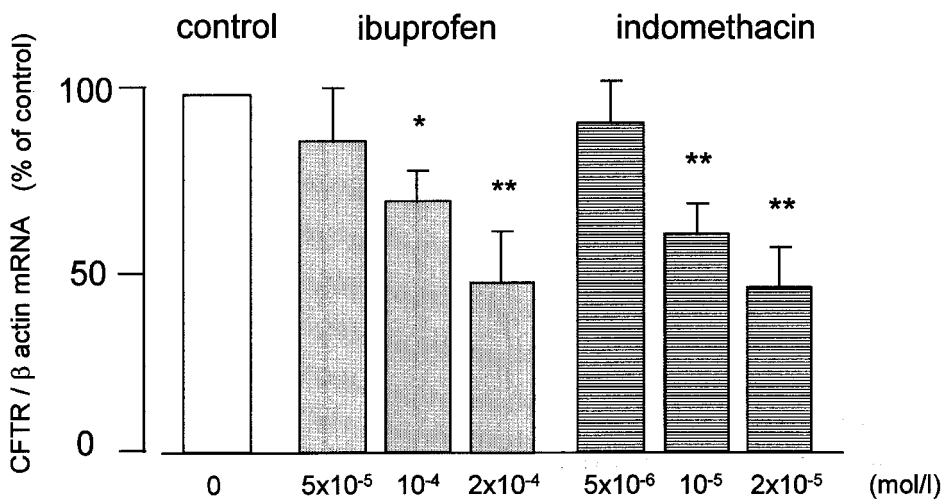

FIG. 5. Concentration dependence of the decay of CFTR transcripts induced by ibuprofen and indomethacin. Northern blots were performed using 15 μg total RNA extracted from cells treated for 24 h with various concentrations of ibuprofen and indomethacin. The cell content in CFTR mRNA is normalized to the content in β-actin mRNA, and the results are expressed as a percentage of the ratio determined on control cells. * $P<0.05$; ** $P<0.01$ ($n = 5$).

+ PGE$_2$

CFTR

β actin

1 2 3 4 1 2 3 4
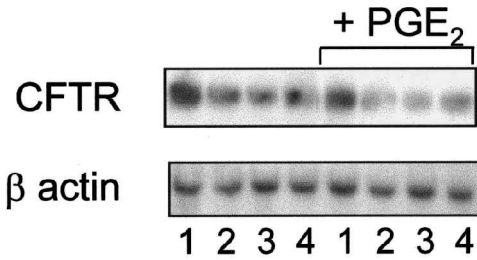
1 : control
2 : + aspirin (10^{-3} mol/l)
3 : + ibuprofen (5x10^{-4} mol/l)
4 : + indomethacin (2x10^{-5} mol/l)

[PGE$_2$] : 10^{-6} mol/l

FIG. 6. Absence of the effect of exogenous PGE$_2$. Typical Northern blot performed using 15 μg total RNA extracted from cells treated for 24 h with aspirin, ibuprofen, and indomethacin in the absence and the presence of exogenous PGE$_2$ (10^{-6} mol/l)

2, row 'aspirin', column 1). Treatment of the cells with the three tested NSAIDs modulated this parameter in various ways (column 4, Table 2). Aspirin decreased it twofold (columns 4 and 7, Table 2), indomethacin was without effect (Table 2, row 'indomethacin', columns 4 and 7), and ibuprofen increased it twofold (Figure 7B and Table 2, row 'ibuprofen', columns 4 and 7).

Discussion

The present study shows that aspirin, indomethacin, and ibuprofen, three nonsteroidal anti-inflammatory drugs widely used in clinical medicine, decrease both the amount of CFTR transcripts and the function of CFTR protein in human T-84 cells.

The decrease in CFTR mRNA does not appear to be toxic, because there was no sign of death in cells incubated for 48 h with the highest concentrations of the drugs used, and because the amount of β-actin mRNA did not change in parallel with that of CFTR mRNA. The high concentrations of aspirin and other drugs required to down-regulate CFTR gene expression raise two issues: first, the possible existence of such an effect during anti-inflammatory treatment;
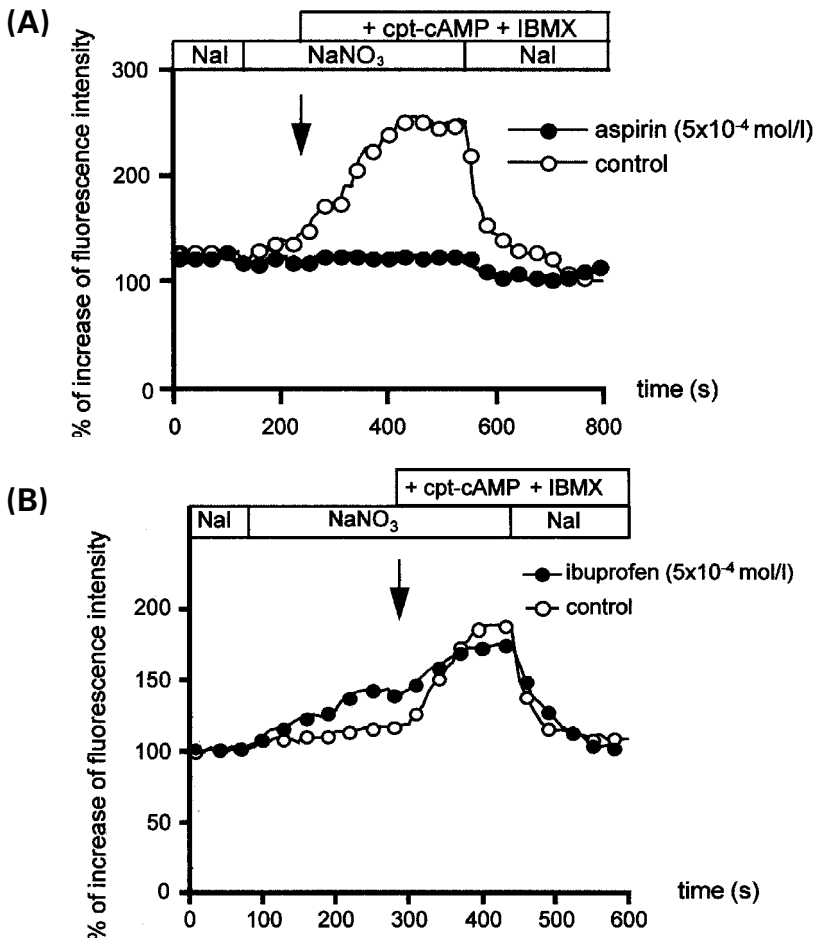

FIG. 7. The fluorescence of intracellular MEQ was measured with respect to time under control conditions and after 48 h of treatment with either 5×10^{-4} mol/l aspirin (A) or 5×10^{-4} mol/l ibuprofen (B). The sequence of buffer substitutions is indicated in the inset.

Table 2. Analysis of MEQ fluorescence in T84 cells

	$\Delta F_{basal}^{ctl}/\Delta t$, untreated cells (column 1)	$\Delta F_{cAMP}^{ctl}/\Delta t$, untreated cells (column 2)	$\Delta F_{cAMP}^{ctl}/\Delta F_{basal}^{ctl}$, untreated cells (column 3)	$\Delta F_{basal}^{test}/\Delta t$, treated cells (column 4)	$\Delta F_{cAMP}^{test}/\Delta t$, treated cells (column 5)	$\Delta F_{cAMP}^{test}/\Delta F_{basal}^{test}$, treated cells (column 6)	$\Delta F_{basal}^{test}/\Delta F_{basal}^{ctl}$ (column 7)
Aspirin (0.5 mmol/l)	0.26±0.04 (10)	2.02±0.38 (10)	7.8	0.10±0.06[a] (10)	0.19±0.03 (10)	1.9	0.38
Ibuprofen (0.5 mmol/l)	0.24±0.04 (6)	2.15±0.14 (6)	8.9	0.58±0.05[b] (6)	1.40±0.15 (6)	2.4	2.41
Indomethacin (2 μmol/l)	0.29±0.02 (8)	1.80±0.18 (8)	6.9	0.25±0.04[c] (8)	0.58±0.07 (8)	2.3	0.86

Untreated cells: $\Delta F_{basal}^{ctl}/\Delta t$, changes in MEQ fluorescence observed in unstimulated cells; $\Delta F_{cAMP}^{ctl}/\Delta F_{basal}^{ctl}$, changes in MEQ fluorescence observed in cells exposed to cAMP cocktail; $\Delta F_{cAMP}^{ctl}/\Delta F_{basal}^{ctl}$, values obtained by dividing values from column 2 by those from column 1. NSAIDS-treated cells for 48 h: $\Delta F_{basal}^{test}/\Delta t$, changes observed in unstimulated cells; $\Delta F_{cAMP}^{test}/\Delta t$, changes in MEQ fluorescence observed in cells exposed to cAMP cocktail; $\Delta F_{cAMP}^{test}/\Delta F_{basal}^{test}$, values obtained by dividing values from column 5 by those from column 4; $\Delta F_{basal}^{test}/\Delta F_{basal}^{ctl}$, values obtained by dividing the values from column 4 by those from column 1. Numbers in parentheses corresponds to the number of independent experiments.
[a]Statistically different from values presented in the line aspirin, column 1, at $P<0.05$.
[b]Statistically different from values presented in the line ibuprofen, column 1, at $P<0.01$.
[c]Not significant from the values presented in the line indomethacin, column 1.

second, the relationship between the modulation of CFTR gene expression and cyclooxygenase inhibition. Plasma concentrations of aspirin as high as those required to modulate CFTR gene expression in our experimental model (over 10^{-4} mol/l) have been reported when the drug is used for sustained periods,[19] so our results may have clinical relevance. However, the concentrations of aspirin, indomethacin, or ibuprofen required to reduce CFTR gene expression in T-84 cells are not consistent with the direct implication of cyclooxygenase (COX) inhibition, whether of the constitutive COX-1 or the inducible COX-2. Aspirin, indomethacin, and ibuprofen are better inhibitors of COX-1 than of COX-2, and thus have a higher ID_{50} for COX-2 than for COX-1; but even their reported ID_{50} for COX-2[20] is at least 10-fold lower than their active concentrations in the present study. Furthermore, the failure of exogenous PGE_2 to prevent the decrease in CFTR gene expression caused by aspirin, indomethacin, and ibuprofen suggests that this effect of the NSAIDs does not involve the inhibition of prostaglandin synthesis. The NSAID concentrations used in the present study are comparable with those used in recent studies of the effects of these drugs on the nucleus. This is the case for indomethacin binding to the peroxysome proliferator-activated receptor γ (PPARγ).[21] High concentrations of aspirin are also required to modulate the effect of the transcription factors such as NFκB, AP1, or HSF,[7–9] which possess specific binding sites in the CFTR promoter,[12] and may thus be involved in the NSAID-induced down-regulation of CFTR gene expression. Further studies will be required to demonstrate the existence of any such regulation of CFTR gene expression by aspirin and its derivatives. Since the transcription factors affected by aspirin are activated during inflammation, this putative mechanism of negative modulation of CFTR expression would also result from an anti-inflammatory effect of the NSAIDs.

The cAMP-stimulated anionic efflux measured by the MEQ assay was also decreased by treating the T-84 cells with aspirin, indomethacin, or ibuprofen. Since the experiments were performed without NSAIDs, this effect of the NSAIDs differed from the immediate inhibition of the ionic transport described in other studies.[2–6] The present data demonstrate that the NSAIDs also exert a delayed action on anionic fluxes. The results showing differences in the concentration dependence of cAMP-regulated anion fluxes and in the modulation of CFTR mRNA production argue against a direct causal relationship between the two effects of NSAIDs. Two points may be made. First, the relative inhibition of cAMP-triggered anion efflux is larger than the relative decrease in CFTR transcripts. This was particularly obvious in cells treated with 5×10^{-4} mol/l aspirin, which no longer responded to cAMP in term of anionic efflux, despite a decrease of only about 30% in their CFTR mRNA content. Second, very few CFTR channels seem to be necessary for this function.[11] In normal tissues, this small number of channels is correlated with a quantity of CFTR gene products too small to be visualized by Northern blotting. Our results therefore suggest that aspirin, ibuprofen and indomethacin have long-acting effects on CFTR function, in addition to their action on CFTR gene expression. The NSAIDs may alter the cytoplasmic processing and/or turn-over of the CFTR protein, or change its potential for activation. These processes are modulated by variations in temperature and by various compounds (butyrate, glycerol) (a review[22]), and the demonstration of their sensitivity to NSAIDs would be particularly interesting in the cystic fibrosis context. Immunoblots of CFTR protein in control cells and cells exposed for 48 h to 0.5 mmol/l aspirin, incubated with radio-labeled methionine then incubated for 2–30 h showed no clear alteration in the time-course of the CFTR protein glycosylation or degradation, but only a general decrease in the intensities of immature and

fully glycosylated CFTR protein bands. The latter were very faint in NSAID-treated cells (result not shown). These results suggest that there is an aspirin-induced decrease in the synthesis of native CFTR protein, but further studies are required to specify the action of aspirin on CFTR protein processing.

A decrease in endogenous CFTR activators caused by NSAIDs cannot be due to altered production of intracellular endogenous cAMP because the MEQ assay was performed with exogenous permeant cpt-cAMP. Other cytoplasmic factors that control CFTR function may also be affected by the NSAIDs. For example, indomethacin acts as an irreversible inhibitor of cAMP-dependent protein kinase in rabbit ileal mucosa,[23] and inhibits phospholipase A2 in rabbit polymorphonuclear leukocytes.[24] Such effects could alter CFTR function directly or indirectly, but are unlikely since there were no changes in cAMP-induced MEQ fluorescence in cells incubated for only 24 h with NSAIDs.

However, the use of the MEQ methodology did reveal that ibuprofen also appears to induce a new cAMP-independent anion conductance, which can be activated in T-84 cells without further stimulation. This effect may correspond to an increased number and/or function of another chloride channel, and thus contribute to the improved clinical status of CF patients treated with ibuprofen.

The present results emphasize the possible diversity of the NSAID effects, since these drugs, which inhibit CFTR activity accutely,[2-6] also appear to produce a delayed decrease in CFTR gene expression and cAMP-stimulated anionic efflux. The delayed decrease in CFTR protein function does not appear to result from a decrease in gene expression alone. The NSAIDs thus appear to have a broad spectrum of action on CFTR, which may reflect a general modification of cell metabolism. This suggest that CFTR acts as a 'housekeeping gene' that can adapt to various physiological or pharmacological disturbances.[25]

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