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Novel short chain fatty acids restore chloride secretion in cystic fibrosis ☆

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Abstract

Phenylalanine deletion at position 508 of the cystic fibrosis transmembrane conductance regulator (ΔF508-CFTR), the most common mutation in cystic fibrosis (CF), causes a misfolded protein exhibiting partial chloride conductance and impaired trafficking to the plasma membrane. 4-Phenylbutyrate corrects defective ΔF508-CFTR trafficking in vitro, but is not clinically efficacious. From a panel of short chain fatty acid derivatives, we showed that 2,2-dimethyl-butyrate (ST20) and α-methylhydrocinnamic acid (ST7), exhibiting high oral bioavailability and sustained plasma levels, correct the ΔF508-CFTR defect. Pre-incubation ($\geqslant$6 h) of CF IB3-1 airway cells with $\geqslant$1 mM ST7 or ST20 restored the ability of 100 μM forskolin to stimulate an $^{125}I^-$ efflux. This efflux was fully inhibited by NPPB, DPC, or glibenclamide, suggesting mediation through CFTR. Partial inhibition by DIDS suggests possible contribution from an additional Cl^- channel regulated by CFTR. Thus, ST7 and ST20 offer treatment potential for CF caused by the ΔF508 mutation.

Keywords: Cystic fibrosis; ΔF508-CFTR; 2,2-dimethyl-butyrate; α-methylhydrocinnamic acid; Iodide efflux; IB3-1 airway cells; Butyrate derivatives; Intracellular trafficking; Chloride channel; Chloride conductance

Cystic fibrosis (CF), an autosomal recessive disease affecting up to 1 of every 2200 Caucasian live births, is a disorder of fluid and electrolyte secretion affecting sweat ducts, airways, vas deferens, and digestive organs such as liver, pancreas, and intestines. This condition is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), a membrane protein functioning both as a chloride conductance and as a regulator for other ion channels, such as the epithelial sodium channel (ENaC) and the outwardly rectified chloride channel (ORCC). Deletion of the phenylalanine at position 508 (ΔF508) is the most common mutation of CFTR, accounting for 75–90% of CF alleles. This mutation produces a misfolded protein that is improperly processed and subsequently fails to be expressed on the cell surface membrane, its site of action. However, when ΔF508-CFTR trafficks to the cell surface following certain manipulations (e.g., culture at 25 °C, treatment with glycerol), cAMP-activated Cl^- conductance is demonstrated, suggesting that the mutated CFTR retains partial Cl^- conductance function [1]. Compounds such as butyrate and 4-phenylbutyrate (4-PBA) correct this trafficking defect in vitro [2,3]. Butyrate and 4-PBA may also enhance CFTR gene transcription. However, the clinical use of sodium 4-PBA (Buphenyl), which is already approved by the FDA for the treatment of urea cycle disorders, is limited by its rapid metabolism, resulting in the requirement for large oral doses for 4-PBA (19 gm/day) and limited long-term therapeutic efficacy [4,5]. We now report that arginine butyrate (AB) and two novel short chain fatty acid derivatives (SCFAD), α-methylhydrocinnamic acid (ST7) and

☆ *Abbreviations:* 4-PBA, 4-phenylbutyrate; AB, arginine butyrate; CFTR, cystic fibrosis transmembrane conductance regulator; CF, cystic fibrosis; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; DPC, diphenylamine-2-carboxylate; ENaC, epithelial sodium channel; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid; ORCC, outwardly rectified chloride channel; SCFAD, short chain fatty acid derivatives; ST20, 2,2-dimethyl-butyrate; ST7, α-methylhydrocinnamic acid.

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2,2-dimethyl-butyrate (ST-20), functionally correct the ΔF508-CFTR defect. AB is a formulation of butyrate already in clinical use for β-globin diseases, while ST7 and ST 20 yield sustained high plasma concentrations following oral administration [6,7].

Materials and methods

Compounds and reagents. Compounds evaluated include 2,2-dimethyl-butyric acid (Lancaster Synthesis, Lancaster, NH), sodium-2,2-dimethylbutyrate (Seres Laboratories, ⩾98.5% purity by gas chromatography), butyric acid (Aldrich, Milwaukee, WI), α-methylhydrocinnamate (T.E. Neesby, Fresno, CA), and 4-phenyl butyrate (Sigma, St. Louis, MO). The structures of these compounds are shown in Fig. 1. Tissue culture medium and supplements and chloride channel inhibitors were from Sigma and $Na^{125}I$ (16 mCi/mg iodide) was purchased from Amersham (Arlington Heights, IL).

Cell culture. Airway IB3-1 cells, compound heterozygotes for the CFTR mutations ΔF508 and W1282X, were used as in vitro model for ΔF508-CFTR correction since the W1282X mutation results in a truncated protein that is not expressed [8]. IB3-1 cells were cultured in keratinocyte growth medium (Clonetics, San Diego, CA) supplemented with bovine pituitary extract (30 μg/ml), human epidermal growth factor (0.1 ng/ml), insulin (5 μg/ml), hydrocortisone (0.5 μg/ml), gentamycin sulfate (50 μg/ml), amphotericin B (50 μg/ml), and $CaCl_2$ (1 mM), and on top of Transwell inserts (Costar, Cambridge, MA). Gentamycin (and amphotericin B contained in the same mixture) was omitted from the culture medium when the effect of aminoglycosides on the W1282X mutation was studied.

After monolayer confluence was reached and before experimental use (~1 week), the medium covering the cells was removed to mimic an air/tissue interface. When the effects of SCFAD were studied, these agents were dissolved in medium, with subsequent adjustment to neutral pH with choline bicarbonate or L-arginine base, and added to both sides of the cells (above and below the insert).

Efflux studies. The use of cellular $^{125}I^-$ effluxes to study the activation of Cl^- conductances has been validated [9]. Transwell membranes supporting confluent monolayers of IB3-1 cells were excised from the insert and washed twice with 1 ml of efflux buffer consisting of (in mM) 140 NaCl, 4.7 KCl, 1.2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 Hepes (pH 7.4). The IB3-1 cells were then loaded with radioactive $^{125}I^-$ by incubating for 45-min at 37 °C with 1.5 ml of efflux buffer containing ~2 μCi/ml $Na^{125}I$ and washed four times with 2 ml of isotope-free buffer. Efflux of $^{125}I^-$ was measured by sequential addition and removal of 1 ml of isotope-free buffer at 15 s intervals for a 5-min period. After one minute to establish baseline efflux, 100 μM forskolin was added to stimulate the cAMP pathway and activate potential CFTR conductance. When indicated, inhibitors were added at the beginning of the experiment (including the baseline monitoring period). The radioactivity of these sequential samples and the radioactivity remaining in the cells at the end of the experiment were measured using a gamma counter (Isodata 120, ICN, Huntsville, AL).

The radioactivity contained in the cells at a particular time point was calculated as the sum of the radioactivities released in subsequent efflux samples and remaining in the cells at the end of the experiment. The efflux rate coefficient for a certain time interval was also calculated using the formula:

$$r = [\ln(R_1) - \ln(R_2)]/(t_1 - t_2),$$

where R_1 and R_2 are the percent of counts initially loaded remaining in the cells at times t_1 and t_2.

Statistics. Unless specified otherwise, results were expressed as means and standard error (in some figures, error bars may not be visible if smaller than data point symbols). Because of variations in the $^{125}I^-$ effluxes between experiments performed on different days, each figure shown and each comparison made refer only to experiments performed on the same day and using cells cultured in parallel; however, they are representative of at least two series of experiments performed on different days. Increases in the efflux rate coefficient were calculated by subtracting the rate coefficient at 1 minute, just prior to the addition forskolin, from the peak efflux rate coefficient at the times designated. Statistical significances of observed differences were determined through unpaired two-tailed Student's *t* test, using the Statview 512+ software program (Brainpower Inc., Calabasas, CA).

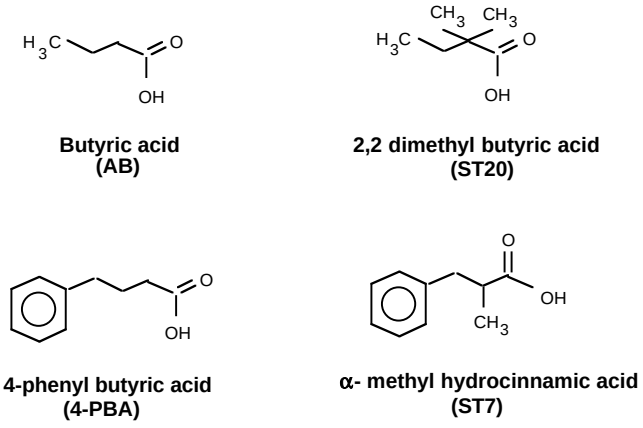

Fig. 1. Chemical structures of butyrate (AB), 4-phenyl butyric acid (4-PBA), α-methyl hydrocinnamic acid (ST7), and 2,2-dimethyl-butyrate (ST20).

Results

Effect of SCFAD derivatives on forskolin-stimulated $^{125}I^-$ efflux from IB3-1 cells

Expression of functional ΔF508-CFTR on IB3-1 cells following SCFAD exposure (2 days with 2.5 mM) was first demonstrated in vitro through the ability of forskolin to stimulate a cAMP-dependent $^{125}I^-$ efflux from these cells (Fig. 2). Consistent with previous reports [2,8], forskolin only stimulated an increase in the $^{125}I^-$ efflux from IB3-1 cells pretreated with 4-PBA, but not from untreated cells. In a similar manner, pretreatment with AB, ST7, or ST20, also restored the ability of forskolin to stimulate $^{125}I^-$ efflux from these cells. There was no statistically significant difference between the forskolin-stimulated increases in efflux rate coefficients following treatment with either 4-PBA, AB, ST7 or ST20.

Table 1 summarizes the ability of additional SCFAD, screened at a concentration of 2.5 mM and following 2 days of exposure, to restore the ability of forskolin to increase $^{125}I^-$ efflux from IB3-1 cells. While other agents were also effective, AB, ST7, and ST20 were selected for detailed studies. AB was selected because it has been administered in a clinical setting for treatment of β-globin disorders and because sodium butyrate has been shown to be effective in correcting the ΔF508-CFTR defect. ST7 and ST20 were selected because they have been shown to have advantageous pharmacokinetic and safety profiles in non-human primate animal studies, which would favor efficient oral administration and feasibility for potential therapeutic use [7].

The dependence of this functional correction on the duration of pre-incubation with SCFAD and on SCFAD

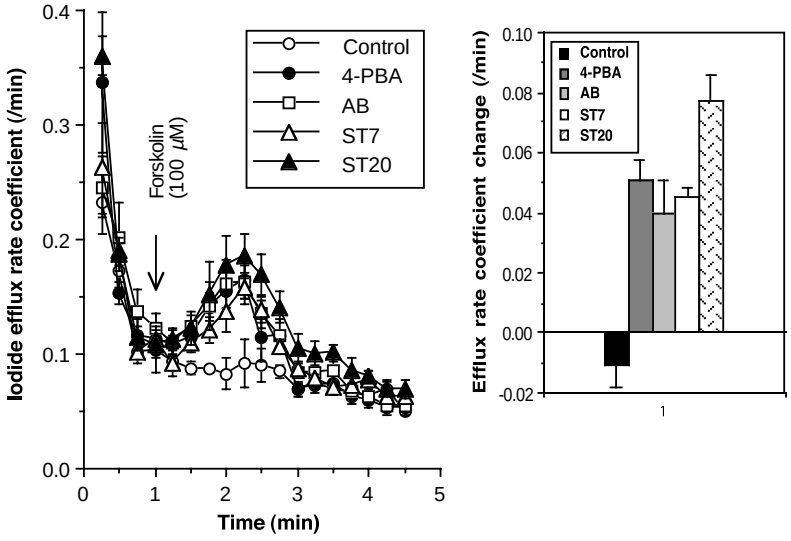

Fig. 2. Effect of SCFAD on forskolin-stimulated $^{125}I^-$ efflux from IB3-1 cells. Confluent monolayers of IB3-1 cells were pretreated for 2 days with 2.5 mM of either 4-PBA (●), AB (□), ST7 (Δ), ST20 (▲), or arginine-containing vehicle (control, ○) and the $^{125}I^-$ efflux stimulated by 100 μM forskolin determined as outlined in the Methods. The mean and SEM from three different experiments are shown for each data point. The inset shows the increases in peak efflux rate coefficients (coefficient at 2.25 min minus coefficient at 1 min) which were, respectively, -0.011 ± 0.007/min, 0.051 ± 0.006/min, 0.040 ± 0.011/min, 0.045 ± 0.003/min, and 0.077 ± 0.009/min, for untreated cells and cells treated with 4-PBA, AB, ST7, and ST20 ($p < 0.05$ for all SCFAD treatments vs. untreated control).

Table 1
Effects of added SCFAD on the $^{125}I^-$ efflux from IB3-1 cells

Positive response	Borderline response	Negative response
3-Methoxycinnamic acid	DL-β-amino butyric acid	α-Methyl phenoxyacetic acid
4-Methoxycinnamic acid	3-(3,4-Dimethoxyphenyl) propionic acid	Caffeic acid[a]
(+/−)-2-Methylbutyric acid	2,2-Dimethyl propionic acid	4-3,(4-Dimethoxyphenyl) butyric acid
Phenoxyacetic acid	Isobutyramide	(3-4-Methylenedioxyl) cinnamic acid
	Levulinic acid	2-Methylhydrocinnamic acid
	3-Phenylbutyric acid	Methyltrans-cinnamate
		1-Naphthylacetic acid
		(4-Oxypentanoic acid) γ-ketovaleric acid
		Sodium butyryl hydroxamate[a]

Confluent IB3-1 cells were treated with 2.5 mM SCFAD for 2 days and the $^{125}I^-$ efflux stimulated by 100 μM forskolin was determined as described in the Methods. The peak change of efflux rate coefficients was calculated by subtracting the baseline efflux at 1 min (just prior to the addition of forskolin) from the peak efflux at 2.5 min (1.5 min following forskolin). A positive response denotes a robust peak $^{125}I^-$ efflux increase that was statistically different from control ($n = 3$, $p < 0.05$ by unpaired two-tailed t test); a borderline response denotes a discernible $^{125}I^-$ increase which did not reach statistical significance; and a negative response denotes no discernible $^{125}I^-$ efflux increase.

[a] Cellular injury as suggested by the detachment of cells from the filter.

concentration was next examined. The effects of treatment for different time periods with AB (A), ST7 (B), and ST20 (C) on the ability of forskolin to subsequently stimulate $^{125}I^-$ efflux are shown in Fig. 3 and summarized in Table 2. For all three agents, statistically significant responses were observed following incubation $\geqslant 3$ h. In all subsequent experiments, IB3-1 cells were treated with SCFAD for 2 days.

The dependence of the corrective effect on the concentration of the SCFAD was also verified, as shown in Fig. 4 and summarized in Table 3. Overall, small increases in forskolin-stimulated efflux were first observed following pre-incubation with SCFAD at concentrations as low as 0.5 mM; these effects reached statistical significance at concentrations $\geqslant 1$ mM, with additional concentration-dependent increases in efficacy at higher concentrations (e.g., effect at 5 mM > effect at 2.5 mM).

Characterization of the forskolin-stimulated $^{125}I^-$ efflux following treatment with SCFAD derivatives

To verify that this $^{125}I^-$ efflux was mediated through Cl^- channels, the effects of different Cl^- channel blockers were assessed. As shown in Fig. 5, the $^{125}I^-$ efflux stimulated by forskolin following pre-incubation with any of the three SCFAD [(A) for AB, (B) for ST7, and (C) for ST20] was totally inhibited by 500 μM glibenclamide, 2.5 mM DPC, or 500 μM NPPB. Partial inhibition was observed with 500 μM DIDS.

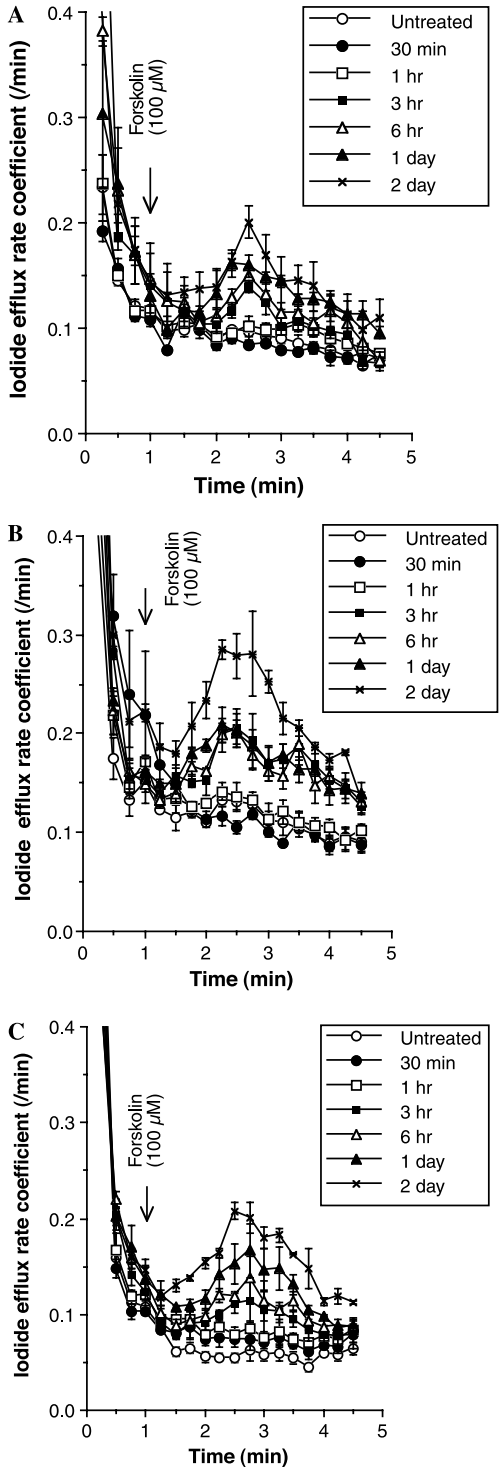

Fig. 3. Dependence of SCFAD effect on duration of pretreatment.Confluent monolayers of IB3-1 cells were pretreated for the indicated lengths of time with 2.5 mM of either AB (A), ST7 (B), or ST20 (C), and the $^{125}I^-$ efflux stimulated by 100 μM forskolin determined as outlined in the Methods. The mean and SEM from three different experiments are shown for each data point.

Because IB3-1 cells are ΔF508/W1282X compound heterozygotes for the CFTR gene, effects of SCFAD on the W1282X mutation rather than the ΔF508-CFTR should be considered. Indeed, read-through of the mutation was observed following treatment with different aminoglycosides [10,11]. The effects of the aminoglycosides, G418, and gentamycin, were therefore compared to the effect of ST20. As shown in Fig. 6, the corrections observed with gentamycin and G418 were rather modest compared to that demonstrated with ST20. At 2.25 min, the peak iodide efflux rate change stimulated by forskolin following 2 day pretreatment with 200 μg/ml G418 was -0.049 ± 0.015/min [vs. 0.013 ± 0.002/min for ST20, and -0.053 ± 0.008/min for untreated control, (A)]. At 2.75 min, the peak efflux rate changes corresponding to pretreatment with gentamycin and ST20 were, respectively, -0.009 ± 0.026/min and 0.039 ± 0.009/min (vs. -0.053 ± 0.001/min for untreated controls). In both instances, statistical differences were only demonstrated with ST20 treatment vs. untreated controls ($p < 0.05$, unpaired two-tailed t test, $n = 3$). When aminoglycosides were combined with ST20, there was no significant enhancement in the response compared to ST20 treatment alone.

Table 2
Dependence of SCFAD effect on duration of pre-incubation

	Peak efflux changes (/min)		
	AB	ST7	ST20
Control	-0.021 ± 0.006	-0.019 ± 0.017	-0.045 ± 0.009
1 h	-0.015 ± 0.001	-0.035 ± 0.017	-0.036 ± 0.008
3 h	-0.003 ± 0.025	$0.051 \pm 0.011^*$	-0.010 ± 0.031
6 h	0.003 ± 0.026	$0.056 \pm 0.020^*$	0.002 ± 0.036
1 day	$0.028 \pm 0.010^*$	$0.038 \pm 0.019^\dagger$	$0.030 \pm 0.015^*$
2 days	$0.056 \pm 0.009^*$	$0.058 \pm 0.016^*$	$0.054 \pm 0.010^*$

IB3-1 cells were pre-incubated with AB, ST7, or ST20, or with arginine-containing vehicle (untreated control) for different time periods and the subsequent $^{125}I^-$ efflux stimulated by 100 μM forskolin was determined, as shown in Fig. 3. The peak changes of efflux rate coefficients, calculated by subtracting the baseline efflux at 1 min from the peak effluxes at 2.5 min (for AB and ST7) and 2.75 min (for ST20), are shown (mean ± SEM). For each SCFAD, the experiments were performed on the same day, using cells grown in parallel; * and † denote statistically significant differences ($p < 0.05$ by two-tailed t test and one-tailed t test, respectively) with the untreated control. Because the different SCFAD were tested separately, results between different SCFAD in this table are not comparable.

Discussion

The identification of CFTR as the protein involved in CF and of ΔF508-CFTR as the most common CF-causing mutation suggests two potential strategies for the treatment of CF: gene therapy and pharmacologic correction of the ΔF508-CFTR defect. While gene therapy is the most elegant and direct approach to a genetic disease, a clinically applicable strategy for CF has not yet been identified. Indeed, problems with efficient gene transfer, stability of the transduced gene, and adverse immunologic responses have hampered advances in this field. Pharmacologic interventions are aimed at activating alternate Cl^- transport pathways (e.g., the Ca^{2+}-activated chloride channel) to bypass defective CFTR chloride conductance, or at correcting of the inability of ΔF508-CFTR to traffic to the cell

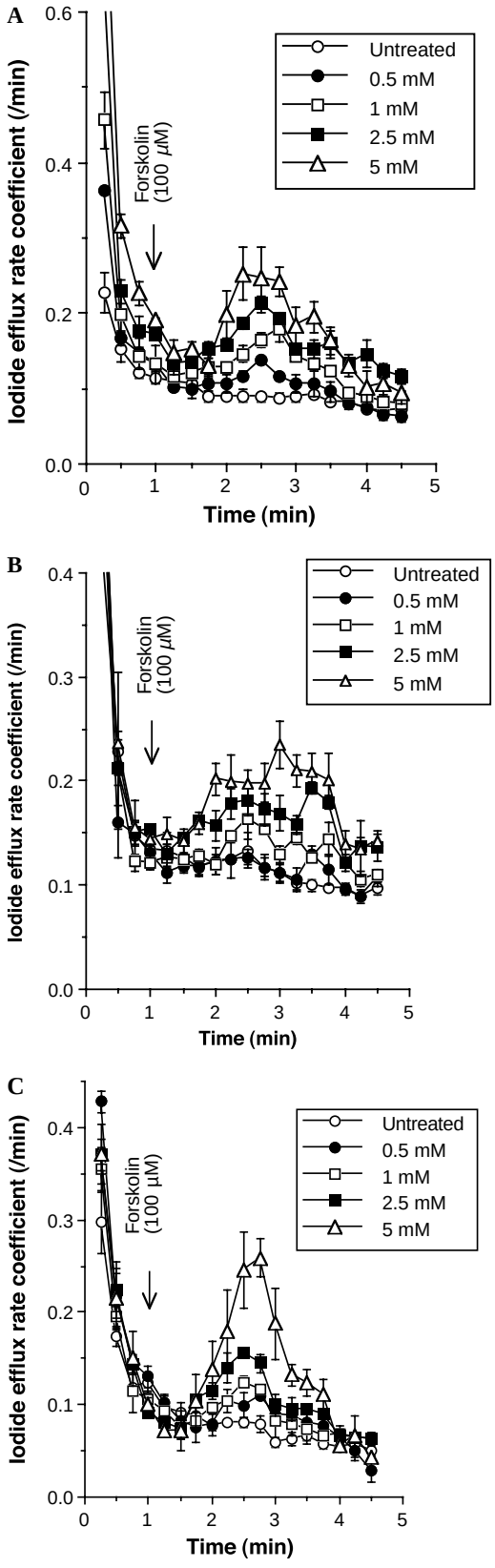

Fig. 4. Concentration-dependence of SCFAD effect. Confluent monolayers of IB3-1 cells were pretreated for 2 days with either AB (A), ST7 (B), or ST20 (C), at the indicated concentrations, and the $^{125}I^-$ efflux stimulated by 100 μM forskolin determined as outlined in the Methods. The mean and SEM from three different experiments are shown for each data point.

Table 3
Concentration-dependency of SCFAD correction

	Peak efflux changes (/min)		
	AB	ST7[a]	ST20
Control	-0.026 ± 0.002	-0.001 ± 0.002	-0.045 ± 0.019
0.5 mM	0.005 ± 0.023	0.004 ± 0.005	-0.021 ± 0.011
1 mM	$0.033 \pm 0.008^{*}$	$0.041 \pm 0.014^{*}$	$0.009 \pm 0.006^{\dagger}$
2.5 mM	$0.040 \pm 0.016^{*}$	$0.039 \pm 0.07^{*}$	$0.053 \pm 0.010^{*}$
5 mM	$0.114 \pm 0.017^{b,*,\%}$	$0.092 \pm 0.029^{*,\%}$	$0.158 \pm 0.027^{*,\%}$

IB3-1 cells were pre-incubated with different concentrations of AB, ST7, or ST20 for 2 days and the subsequent $^{125}I^-$ efflux stimulated by 100 μM forskolin determined, as shown in Fig. 4. The peak changes of efflux rate coefficients, calculated by subtracting the baseline efflux at 1 min from the peak effluxes at 2.5 min (for AB), 2.5–3.5 min (for ST7), and 2.75 min (for ST20), are shown (mean ± SEM). For each SCFAD, the experiments were performed on the same day, using cells grown in parallel; because the different SCFAD were tested separately, results between different SCFAD in this table are not comparable. $p < 0.05$: *vs. control by unpaired two-tailed t test, †vs. control by unpaired one-tailed t test, $^{\%}$vs. 2.5 mM by unpaired two-tailed t test.

[a] Peak effluxes used occurred at 2.5 min for 1 mM, 3.5 min for 2.5 mM, and 3 min for 5 mM.

[b] Peak effluxes used occurred at 2.25, 2.5, and 2.75 min.

surface. Of the several manipulations that correct this trafficking defect in vitro, some are not readily clinically applicable (e.g., hypothermia, glycerol, and dimethylsulfoxide), while others are not practical due to high dose requirements (e.g., 4-PBA) [1,4]. Identification of additional agents that correct the ΔF508-CFTR trafficking defect therefore remains compelling.

Accordingly, we evaluated a panel of SCFAD for activity in correcting the ΔF508-CFTR defect and selected for further studies three compounds which restored forskolin-stimulated $^{125}I^-$ efflux and exhibited favorable properties regarding therapeutic potential in CF. AB is already in clinical evaluation for treatment of hemoglobin disorders; minimal side effects have been observed with this agent [6,12]. The use of L-arginine instead of sodium as the cation for butyrate allows administration of large doses of butyrate without an accompanying sodium load. However, like most butyrates, AB has a short half-life, necessitating prolonged (6–10 h) intravenous infusion. AB was included in this report mainly as a positive control (as was 4-PBA). On the other hand, it is possible that clinical effect may not require sustained concentrations of AB. Indeed, short infusions of AB (pulse-therapy) produced greater benefit than continuous infusion in the treatment of hemoglobin disorders [12]. In addition, the small effect observed with 0.5 mM of AB may not have reached statistical significance because of a type II error, and it is possible that this small effect could be sufficient for clinical benefit. Indeed, neither pulmonary nor gastrointestinal CF symptoms are observed in patients with congenital absence of the vas deferens, in whom CFTR mRNA is only 10% of normal [13].

In this report, the in vitro effects observed with either 4-PBA, AB, ST7, and ST20 were equivalent. However, both ST7 and ST20 provide longer plasma half-lives and high oral bioavailabilities (>90%). As detailed in a recent

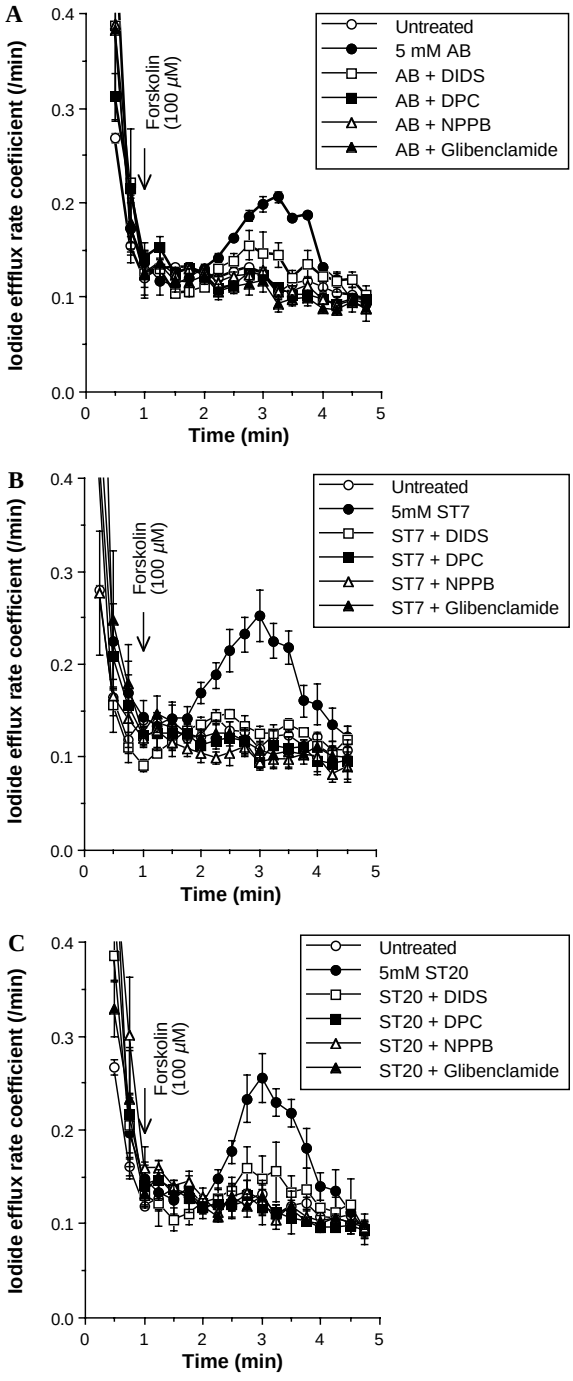

Fig. 5. Inhibition of forskolin-stimulated $^{125}I^-$ efflux following SCFAD treatment by inhibitors of chloride channels. Confluent monolayers of IB3-1 cells were pretreated for 2 days with either 5 mM AB (A), ST7 (B), or ST20 (C), and the $^{125}I^-$ efflux stimulated by 100 μM forskolin in the presence or absence of 500 μM glibenclamide, 500 μM NPPB, 2.5 mM DPC, or 500 μM DIDS, determined as outlined in the Methods. The means and SEM from three different experiments are shown.

report [7], following an oral administration of 100–200 mg/kg of these SCFAD, millimolar plasma concentrations were sustained in juvenile baboons for more than 2 h for ST7 and 8 hours for ST20. These concentrations were effective in vitro in correcting defective ΔF508-CFTR expression and function, as evidenced by restoration of

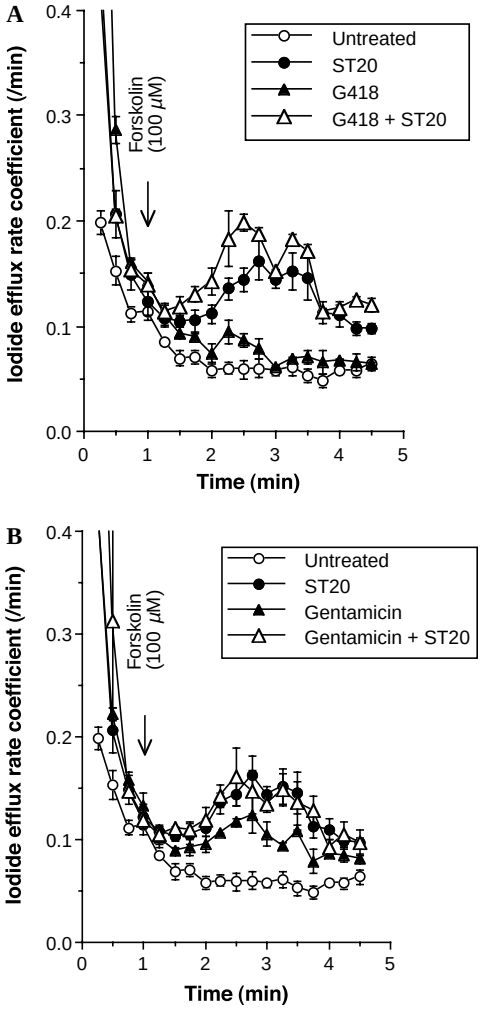

Fig. 6. Comparison of ST20 and aminoglycoside treatment on forskolin-stimulated $^{125}I^-$ efflux. Confluent monolayers of IB3-1 cells were pretreated for 2 days with 2.5 mM ST20, 200 μg/ml G418 (A), or 200 μg/ml gentamycin, (B) alone or in combination, and the $^{125}I^-$ efflux subsequently stimulated by 100 μM forskolin determined as outlined in the Methods. The mean and SEM from three different experiments are shown for each data point. The experiments in this figure were generated together but separated into two panels for clarity.

cAMP-stimulated Cl^- channel activity. These concentrations are also ~10-fold higher than the maximal plasma levels of AB of 0.02–0.05 mM that were achieved with continuous intravenous administration of this drug at 500–2000 mg/kg/day [6]. The pharmacokinetic properties of ST7 and ST20 also compare favorably with 4-PBA. Indeed, when 4-PBA was administered orally to patients with sickle cell anemia or malignancy, for up to 20–36 g/day in three divided doses, the plasma concentrations remained above 1 mM for no more than 2 h after each dose of 7–12 gram/day [14,15]. In the dose-escalation study with adult CF patients, while 30 g/day was the maximal dose tolerated, the practical daily dose was 20 g, taking into account adverse reactions and safety profile. In contrast, since similar human plasma levels are reached with only 20% of a juvenile baboon dose, in an adult human weighing 50–60 kg, it is projected that 1–1.5 g ST7 or ST20 will

suffice to achieve the effective millimolar plasma concentrations.

As the salts of ST7 and ST20 are highly soluble in aqueous solutions, the targeted plasma concentrations of ST7 or ST20 can be reached using tolerable dose volumes, e.g., 5 ml of a 200 mg/ml solution or suspension for a 60 kg adult, once or twice per day. By comparison, the daily dose of 20 g of 4-PBA requires 40 tablets of 500 mg each [5]; this large number of pills may hinder patient compliance [14]. Importantly, as assessed by daily monitoring of behavior, appetite, and activity, thrice weekly monitoring of complete blood count, and weekly monitoring of serum chemistry and physical examination, no toxicity was observed when ST7 and ST20 were administered to baboons [7].

Of note, unlike butyrate and 4-PBA, neither ST7 nor ST20 inhibits histone deacetylase [16]. Because generalized inhibition of histone deacetylase typically causes cell cycle arrest, inhibits cellular growth, and stimulates apoptosis, the more specific action of ST7 and ST20 may curtail such potential long-term adverse effects associated with histone deacetylase inhibition (e.g., inhibition of pulmonary epithelial cell growth). Of note, while a recent report suggests that inhibition of histone deacetylase may up-regulate the expression of splicing factors and correct the CFTR 3849+10 kB C → T splicing mutation [17], this effect does not appear to be operative in ST7 and ST20 correction of ΔF508-CFTR.

The uses of IB3-1 cells to study the functional correction of the ΔF508-CFTR defect and of forskolin-stimulated $^{125}I^-$ efflux to measure CFTR activity are well established [2,9]. With this model AB, ST7, and ST20 all corrected the ΔF508-CFTR defect in a concentration-dependent manner, as they all restored cAMP-activated Cl^- conductance activity to IB3-1 cells. Of note, this correction required a pre-incubation period of at least 3 h, consistent with an effect on ΔF508-CFTR trafficking or synthesis; direct activation of CFTR should have a faster onset of action.

Induction of CFTR expression by SCFAD in IB3-1 cells is most likely achieved through correction of ΔF508-CFTR [2,3]. However, because these cells are compound heterozygotes for W1282X, a premature CFTR stop mutation that can be suppressed with aminoglycosides, a possible SCFAD effect on this mutation was considered [11]. Correction with gentamycin or G418 was much smaller than the one observed with ST20. This very modest response did not allow us to evaluate for an additive effect of combined ST20 and aminoglycosides. However, the much smaller response observed with W1282X-CFTR readthrough, as induced by aminoglycosides, suggests that the larger response observed with SCFAD is mediated via ΔF508-CFTR correction, as previously asserted [2,3].

Of interest, the forskolin-stimulated $^{125}I^-$ efflux observed following treatment with SCFAD is not only inhibited by glibenclamide and DPC, it is also partially inhibited by DIDS. Full inhibition by glibenclamide and DPC is consistent with the mediation of this $^{125}I^-$ efflux by CFTR. However, CFTR is resistant to DIDS and partial inhibition by DIDS suggests that this efflux may be mediated by an additional Cl^- channel regulated by, but distinct from, CFTR. The outwardly rectified chloride channel (ORCC) is the most likely candidate for this channel, as it is expressed on respiratory cells and is closely regulated by CFTR [18]. Indeed, in IB3-1 cells, the ORCC is functionally silent; upon correction of CFTR expression, its activity is restored, as evidenced by a cAMP-activated and DIDS-sensitive outwardly-rectified Cl^- conductance [3,11]. Thus, it appears that the SCFAD studied in this report not only restored the function of CFTR as a Cl^- conductance, they also restored the function of CFTR as a regulator of other ion transport pathways, such as the ORCC. We should caution, however, that, the Cl^- inhibitors used may not be specific enough for conclusive classification; only electrophysiologic studies will unequivocally define the respective contributions of the CFTR and ORCC to the $^{125}I^-$ efflux observed.

Of note, while ΔF508-CFTR is a functional Cl^- channel, it is not as efficient as wild-type CFTR, exhibiting a shorter open probability [19]. This impairment can be corrected and conductance optimized with phosphatase inhibitors, such as xanthines [3,20] or with flavonoids, such as genistein [21]. Such agents may potentiate the small correction obtained with low concentrations of SCFAD into a clinical response at even lower concentrations of SCFAD. In summary, of multiple SCFAD tested, we have identified two compounds which restore the function of ΔF508-CFTR defect and exhibit favorable pharmacokinetic properties for application as oral therapeutics, at projected doses more likely to be practical for chronic clinical use in human patients. Further in vivo and clinical studies are warranted to evaluate a potential role for these agents in the treatment of CF. In addition, the activity of these agents in other similar disorders of protein misfolding, processing, and trafficking (such as α_1-antitrypsin deficiency, osteogenesis imperfecta), should also be explored for potential therapeutic application [22].

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